THE EFFECTS OF DIFFERENTIAL MATERNAL ENVIRONMENTS PRIOR TO PREGNANCY ON FUTURE OFFSPRING IN HOODED LISTER RATS.

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ABSTRACT

Central to any understanding of the *nature* of an organism is the examination of the relative contributions of heredity and environment to its development. Set within this framework are the literatures studying the environmental forces which interact with biological predispositions to produce the mature individual (Shaffer 1985). One such area of research which has provided evidence that the environment has a beneficial impact on an animal's neurochemistry, neuroanatomy and behaviour is the environmental enrichment literature (Rosenzweig 1984; Renner and Rosenzweig 1987; Rose 1988) where animals reared in socially and perceptually stimulating environments are compared with their littermates raised in impoverished environments.

Within this literature are a handful of studies which suggest that these beneficial effects are not only confined to those animals *directly* exposed to an enriching environment but also can be passed across generations. It is this intergenerational research which provides the focus of this thesis in which the effects of exposing female rats to differential environments prior to pregnancy on successive generations were investigated in Hooded Lister rats.

Chapter one, the introduction to this present work, provides a historical background to the investigation of early experience, enrichment and its effect on the brain and reviews those few studies which have investigated the results of maternal enrichment on the offspring generation. Enrichment as an environmental manipulation has been extensively researched and those studies investigating the behavioural consequences of exposing animals directly to Enriched (EC) and Impoverished (IC) conditions are reviewed in chapter two, to provide a profile against which to compare the offspring generations investigated in this thesis.

The impact of intergenerational effects has of course been explored using manipulations other than enrichment. Indeed, it is now well established that various kinds of stressors imposed upon females of different species can affect both the physiology and behaviour of their offspring (Joffe 1969b; 1978; 1982; Thompson and Grusec 1970; Archer and Blackman 1971). Chapter three of this thesis provides an overview of the literature investigating the effects of manipulation of

the maternal generation either prior to pregnancy, during pregnancy or postnatally on offspring and grandoffspring generations emphasising the diversity of manipulations other than enrichment that have been employed.

Following chapter four, which describes the general methodology employed in this thesis, with details of the breeding programmes, the environmental manipulations and behavioural test apparatus used, are the four experimental studies designed to investigate the effects of enrichment on successive generations. In particular, chapter five (study one) provided a profile of animals exposed directly to environmental enrichment, impoverishment and standard housing (SC) against which to compare future generations' behavioural patterns. Futhermore, this chapter also tested the efficacy of the enriched environment employed in this thesis, best described as a Superenriched environment (SEC) in male, female and postpartum female rats. The inclusion of the latter group was to ensure that the commonly found enrichment effects would continue postpartum despite undergoing pregnancy and litter-rearing.

Moving on to successive generations, chapter six (study two) explored the effects of differential maternal environments prior to pregnancy on offspring and grandoffspring behaviour. Animals were put through a battery of tests to investigate their activity, perceptual and learning performances. From this work qualitatively different behavioural profiles were observed in both the offspring and grandoffspring of the three maternal conditions. Possible causes for the observed performance differences were discussed and it was suggested that they might reflect amongst other things, different learning capacites between the groups or differential arousal and/or stress levels. The last two studies of this thesis were designed to investigate these postulated causes further. Chapter seven (study three) analysed the effects of differential maternal environments prior to pregnancy on offspring performance in the Hebb-Williams maze and in an operant conditioning task, whilst chapter eight (study four) considered the hypothesis that offspring of SEC, SC and IC dams are differentially aroused, by artificially manipulating arousal levels with *d*-amphetamine sulphate.

In the final chapter of this thesis (chapter nine) the main findings of the four studies are summarised and possible causes of the intergenerational transfer of effects discussed. In addition, the individual experiments are critically assessed and avenues for future research suggested.

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CHAPTER ONE: GENERAL INTRODUCTION

Were man able to trace every effect to its cause, he would probably find that the virtue or the vice of the individual, the happiness or misery of the family, the glory or the infamy of a nation had their sources in the cradle, over which the prejudices of a nurse have presided. The years of infancy are those in which the chains of virtue or vice are generally forged, for in proportion to the length of time any idea occupies the mind so does it acquire strength and produce conviction.

Samuel H. Smith 1796.

1:1 HISTORICAL BACKGROUND TO THESIS

According to Jerome Kagan (1979) the infant has a special symbolic meaning in all societies because it marks a beginning. Furthermore in the West "where origins are sacred, the infant is awarded a set of unique qualities, the most important of which is the capacity to be changed permanently by experience" (p13).

Historically, the importance of experience as a major determinant of the "nature" of an organism can be traced back to the Classical philosophers and in particular to Aristotilean heuristics (Hall, Perlmutter and Lamb 1982). In contrast to this "environmentalist" viewpoint, the Platonic version of determinism (Plato: The Republic) stressed the importance of *innate* differences in aptitudes amongst human beings and that these individual differences should be recognised. This "nativist" position became increasingly reinforced by the Church, such that by Medieval times, humans were considered to be sinful and corrupt by nature and that this "original sin" could only be redeemed through Christian salvation (Santrock and Bartlett 1986). More recently, towards the end of the seventeenth century, John Locke (1690) argued that the neonate arrived in the world with an empty mind, a *Tabula Rasa*, on which experience carved out the various qualities which we ascribe to humanity. By the late eighteenth century, however, a new version of nativism had been proposed by Jean-Jaques Rousseau (1762) a French philosopher reacting to the prevalent idea that human beings are inherently wicked. He revived the Platonic view that children are born innately good, and in his book "Emile" suggested that the child should be permitted to grow naturally, with little or no monitoring, such that inborn propensities could guide a healthy development.

Given these philosophical traditions, it is not surprising that a continuing debate in modern psychology concerns the relative contribution of heredity and environment to the development of an organism, although as Harris (1971) has pointed out, one by-product of this history has been to divide people into patterns of thinking or ideology, creating an unecessary or false dichotomy. Terminology has varied from time to time, nature versus nurture, nativism versus cultural realism, genetics versus social controls, maturation versus learning or innate traits versus aquired characteristics (Thomas 1985), but the basic issue has remained the same: how do inborn factors compare with environmental factors in determining the nature of an organism?

Early in this century, the question of causality was predominant, with Wiggam (1923) for example arguing that the genetic makeup of an animal was the determining factor in development.

> "Heredity, and not environment is the chief maker of man... The differences amongst men are due to the differences in the germ cells with which they are born" (p42).

The opposing viewpoint, that of the nurturist can be exemplified by Watson (1925), who wrote:

> "Give me a dozen healthy infants, well formed and my own specified world to bring them up in and I'll guarantee to take any one at random and train him to become any type of specialist I might select- doctor, lawyer, artist, merchant, chief and yes, even beggar-man and thief, regardless of his talents, penchants, tendencies, abilities, vocations, and race of his ancestors. There is no such thing as an inheritance of capacity, talent, temperament, mental constitution and behavioural characteristics" (p82).

Few contemporary psychologists, however, would endorse either of these radical points of view. Indeed, the current consensus of opinion is that the relative contributions of nature and nurture depend upon the aspect of development in question. Complex human attributes such as temperament, intelligence and personality are the result of the *interaction* of biological predispositions

and environmental forces (Shaffer 1985).

Furthermore, on the question of causality, the debate has progressed from *which one* to *how much* and subsequently to *in what way* (Anastasi 1958). Indeed Denenberg (1982) has argued that the philosophical basis of causal attribution is no longer valid and "now acts as a major intellectual and emotional barrier blocking attempts to gain an understanding of the deeper structure underlying developmental processes" (p78). He points out that a rigid adherence to simple cause-effect thinking prevents us from advancing conceptually and argues that a new paradigm, General Systems Theory (Bertalanffy 1969) should replace the Aristotilean linear cause-effect model. The key to the more complex systems model has been dealt with from a philosophical perspective by Bunge (1979), in his book "A World of Systems" and relies on the simple assumption that every thing interacts with other things. According to Denenberg (1982) "it is now time for researchers to deal with interactions at a conceptual level as an inherent property of systems rather than to think of them simply as statistical nuisances resulting from a particular type of experimental design involving the analysis of variance" (p80).

One area of research in which both the linear cause-effect model and the more sophisticated interactionalist approach have been applied to gain insights into the processes underlying development, is the animal literature investigating the effects of early experience on later behaviour (Denenberg 1982). Within this field two factors have repeatedly surfaced as having immense impact: firstly the fundamental importance of the *type* of experience typically manifest as the "experimental manipulation" and secondly the timing of the experience in the organism's life. Set within this literature and of particular relevance to the present thesis are the growing numbers of reports that suggest that an animal's behaviour can be influenced by manipulations imposed upon its mother (Joffe 1969b; Archer and Blackman 1971; Joffe 1978) and some few reports that have extended the notion of early experience across generations (Denenberg and Rosenberg 1967; Thompson, Watson and Charlesworth 1962; Ader and Belfer 1962b; Ressler 1966; Wehmer, Porter and Scales 1970; Lane and Hyde 1973). It is this latter theme which is central to the cur-

rent work and which will be considered in section 1:6 of this introduction. Firstly, however, the importance of early experience will be considered, as it is from this literature that the present work, in which the effects of differential maternal environmental experience *prior* to pregnancy on offspring behaviour are explored, can best be positioned.

1:2 THE IMPORTANCE OF EARLY EXPERIENCE

It is now well established that early experiences can have a profound effect on an organism's development and important consequences for future behaviour (Thompson 1968; Thompson and Grusec 1970; Denenberg 1972; Bond and Joffe 1982). Indeed one commonly held view is that the experiences of infancy produce a set of dispositions that have a continuous influence throughout life, implying that the effects of early experience are difficult to alter. This proposition has its roots in the philosophical traditions described above, as well as being influenced by psychoanalytic theory and data from animal laboratories. With respect to the latter both comparative psychologists and ethologists have alerted the scientific community with demonstrations of dramatic and apparently irreversible effects of early experience in animals, some of the most impressive examples coming from the experiments on imprinting (Spalding 1873; Heinroth 1911; Lorenz 1935; 1937) and the production of abnormal behaviour in rhesus monkeys raised with inanimate wire "mothers" (Harlow 1958; 1960). More recently, however, there has emerged some evidence to suggest that the infant is responsive to change and that the effects of early experience are reversible under proper environmental conditions (Kagan 1979). For example, as early as 1959 Denenberg was commenting on the pervasiveness of interactions between early and late experiences, leading him to conclude that "the data were not consistent with the hypothesis that the effects of early experience are irreversible" (Denenberg 1982, p82). Whether the effects of early experience are permanent or are subject to modification by later experiences, although a fascinating debate in its own right, has relevance to the present work only in that it serves to illustrate the importance of "nurture" in the development of the organism.

Within the context of early experience, one area of research which has had an important impact is the considerable literature on differential environmental experience. Indeed, over the last four decades it has been consistently demonstrated that exposing an animal to either an *enriched environmental condition* (EC) in which typically a group of 10 to 12 animals is placed in a relatively large cage with various objects (Rosenzweig and Bennett 1969) or to an *impoverished environmental condition* (IC) where animals are housed in isolation with no social or perceptual stimulation [1], can affect its behaviour, neuroanatomy and neurochemistry (Rosenzweig, Krech, Bennett and Diamond 1968; Rosenzweig, Bennett and Diamond 1972c; Bennett 1976; Greenough 1976; Rosenzweig and Bennett 1976; 1977; 1978; Walsh 1980; Walsh 1981a; Rosenzweig 1984; Renner and Rosenzweig 1987). As this area of research provides the starting point for the present thesis, the historical background and implications of differential environmental experience will be discussed in more detail in the following section.

1:3 HISTORY AND CONSEQUENCES OF EC/IC

The first report of the *behavioural* consequences of differential environments was in a paper given by D.O. Hebb (1947) at a symposium on learning, entitled "The effects of early experience on problem solving at maturity". He noted that seven rats reared at home as pets, with much of their time spent outside their cages, were superior to their littermates reared in laboratory cages, when tested in a Hebb-Williams maze. He concluded that there was a lasting effect of infant experience on the problem-solving of the adult rat. Since 1947, there have been many reports of superior EC perfomance in the Hebb-Williams maze (Davenport 1976) although the notion that this reflects a learning superiority per se has been questioned (for example: Woods, Ruckelshaus and Bowling 1960; Woods, Fiske and Ruckelshaus 1961; Dell and Rose 1986). A variety of tests linked with several types of learning paradigm, other than maze performance, have also been reported in the literature, including discrimination learning, reversal learning, passive and active avoidance learning, as well as operant conditioning (Davenport 1976; Renner and Rosenzweig

[1] For a full description of these environments the reader is refered to chapter four.

1987). It is clear from the results of these experiments that the effects of enrichment are task and response dependant (Lamden 1985). In addition to learning, a variety of other behaviours has also been examined including exploratory behaviour, motor behaviour, sensory capacity, arousal and play behaviour. As the present research is concerned with the consequences of enrichment of one generation on the *behaviour* of subsequent generations, the findings reporting behavioural effects in animals exposed to EC and IC provide the basis for the literature review in chapter two.

Paralleling the behavioural changes in animals exposed to differential environments have been numerous studies which have reported effects manifesting themselves at the neuroanatomical and neurochemical levels. Enrichment can thus be seen as a *non-invasive* method of exploring the relationship between brain and behaviour and has opened up a valuable avenue of research with implications for understanding the localisation of higher order functions in non-lesioned brains. This impact of enrichment on the brain is fundamental to the interest that has been generated by the effects of differential environments over the years and as such, is of relevance to the present work. The main findings from this literature will be briefly overviewed in the following section.

1:4 ENRICHMENT AND THE BRAIN

As the main thrust of the present thesis is *behavioural* in nature, this overview of the neuroanatomical and neurochemical effects of environmental enrichment and impoversishment aims to give a flavour of the main findings, rather than being a comprehensive review. For a fuller exposition of this work the reader is referred to Rosenzweig, Bennett and Diamond (1972c); Bennett (1976); Rosenzweig and Bennett (1978); Walsh (1980); Jones and Smith (1980); Walsh (1981a); Rosenzweig (1984); Renner and Rosenzweig (1987); Bedi and Bhide (1988).

The notion that an animal's training or experience might alter its brain can be traced back to the eighteenth century. For example, Michele Malacarne (cited in Rosenzweig 1971; Rosenzweig, Bennett and Diamond 1972c) examining the hypothesis that changes occur in brain anatomy

as a result of experience, gave extensive training to one member of several pairs of different animals and no training to the other. Effects of training, he concluded, could be seen in the cerebellum, there being more folds in the cerebellum of the trained animals than the untrained animals. During the nineteenth century, however, lack of adequate experimental and statistical techniques precluded a clear demonstration of the effects of experience on the *human* brain [2]. By the begining of the twentieth century, it was generally thought that the brain could not be measurably altered by experience. In 1953, however, a multi-disciplinary team at the University of California (Berkeley) started to search for relations between naturally occuring differences in brain chemistry and differences in learning ability in *rats* (Rosenzweig, Krech and Bennett 1960; Krech, Rosenzweig and Bennett 1962; Rosenzweig 1964). After considerable study of the effects of brain chemistry on behaviour, they extended their research to see whether there might be an inverse relation, namely whether behaviour affected brain chemistry and found that there was. When comparing brains of littermates kept under enriched or restricted conditions for eighty days after weaning, they found clear effects on brain chemistry (Krech, Rosenzweig and Bennett 1960). In addition to the neurochemical findings, clear anatomical differences also emerged between the groups (Rosenzweig, Krech, Bennett and Diamond 1962).

Since 1962 there have been numerous reports of neuroanatomical brain changes at both the macro and micro anatomical level in the literature, as well as reports of neurochemical changes resulting from different experiences. Considering first the macro-neuroanatomical effects, differential experience has been found to cause small but statistically significant alterations in brain weight with EC animals having on average a 4% heavier cortex than their IC counterparts (Rosenzweig, Krech, Bennett and Diamond 1962). Since this first report, over 80 papers have been published in the literature which have confirmed and extended the original brain weight findings (Rosenzweig, Bennett and Diamond 1972c; Bennett 1976; Rosenzweig and Bennett 1978; Renner and Rosenzweig 1987). Typically enriched rats have a significantly heavier cerebral cortex than impoverished and standard colony animals after both 30 and 80 days exposure to the differential

[2] A fact which is still true today, despite the advances made in brain scanning apparatus.

environments. The difference in cortical weight is not a product of uniform increases in weight across the whole cortex, however, but reflects regional differences in the effects of enrichment such that the greatest magnitude of environmentally induced change is found in the occipital cortex, other areas of the cortex (somesthetic, dorsal and ventral) showing considerably smaller but nevertheless significant differences. It is not yet clear why EC-IC cerebral differences are larger in the occipital cortex than elsewhere in the brain, but since this effect occurs even if the experiment is run in total darkness, or if the rats are blinded (Rosenzweig, Bennett, Diamond, Wu Slagle and Saffran 1969) it might be that the occipital cortex is best seen as an intersensory area (Renner and Rosenzweig 1987) and that EC-IC differences in the occipital cortex are not primarily visual in nature.

One interesting feature of these EC-IC differences is that the cerebral effects vary as a function of both the duration of differential experience and age at which it occurs. For example, exposure to EC-IC for one or three days begining at weaning does not produce significant differences in brain weights, but by four days, highly significant effects are found (Rosenzweig and Bennett 1977; 1978). With starting ages of 60 days or more, clear effects are only found after 15 days experience. Attempts to have also been made to determine the minimum period required to produce cerebral effects (Rosenzweig, Love and Bennett 1968; Ferchmin and Eterovic 1980; 1986) with effects occuring in periadolescent rats exposed to EC for as little as ten minutes a day for four days. Although the shortest periods of enrichment only appear to affect young animals, there is no "critical period" for enrichment, as both very young and very old rats are susceptible to environmental influences (Malkasian and Diamond 1971; Riege 1971; Cummins, Walsh, Budtz-Olsen, Konstantinos and Horsfall 1973; Rosenzweig and Bennett 1978).

Coupled with the publications of an increase in EC cortex when compared with their IC littermates, have been several reports of a decrease in EC subcortex (Rosenzweig, Krech, Bennett and Diamond 1962; Rosenzweig, Bennett and Diamond 1972; Bennett 1976; Rosenzweig and Bennett 1978) reflecting the fact that these animals have a lighter terminal body weight (brain weight

varies with body weight). These subcortical effects vary with age and duration of experience, but typically parallel the percentage difference in terminal body weights.

Following the initial findings of the brain weight changes in animals exposed to differential environments (Rosenzweig, Krech, Bennett and Diamond 1962) anatomical examinations of the thickness of cerebral cortex were initiated (Diamond Krech and Rosenzweig 1964; Diamond, Law, Rhodes, Lindner, Rosenzweig, Krech and Bennett 1966; Diamond 1967) to ascertain whether the change in brain weight reflected changes in bulk or density of the tissue. It is now clear from the studies measuring the thickness of the cortex that it is the bulk rather than the density of the cortex which is altered by environmental experience and that, as with the weight findings, it is the occipital cortex which consistently produces the largest differences. Both effects of age and duration of exposure have also been studied and resultant patterns of cortical changes mapped out. The smallest duration of experience that has been found to affect depth of cortex is four days exposure (Diamond, Ingham, Johnson, Bennett and Rosenzweig 1976) whilst greatest effects have been found in animals placed in modified enriched environments in the pre-weaning period (Malkasian and Diamond 1971) [3].

As well as brain weight and cortical depth measures, the effects of enrichment on width and length of the cerebral cortex and on various hippocampal dimensions have also been reported in the literature. In 1968, Altman, Wallace, Anderson and Das reported that exposing animals to differential environments from weaning for three months, significantly affected the length of the cerebrum in favour of the EC animal. However no differences were found between the groups in width of cerebral hemispheres [4]. In 1969, however, Rosenzweig and Bennett reported that they

[3] For a fuller analysis of cortical depth measurements the reader is referred to Diamond, Krech and Rosenzweig 1964; Diamond, Law, Rhodes, Lindner, Rosenzweig, Krech and Bennett 1966; Diamond, Lindner and Raymond 1967; Rosenzweig, Bennett, Diamond, Wu, Slagle and Saffran 1969; Walsh, Budtz-Olsen, Penny and Cummins 1969; Bennett, Rosenzweig and Diamond 1970; Diamond, Johnson and Ingham 1971; Malkasian and Diamond 1971; Diamond, Rosenzweig, Bennett, Lindner and Lyon 1972; Walsh, Cummins, Budtz-Olsen and Torok 1972; Diamond, Lindner, Johnson, Bennett and Rosenzweig 1975; Diamond, Ingham, Johnson, Bennett and Rosenzweig 1976; Diamond 1976; Diamond, Johnson, Mizono, Ip, Lee and Wells 1977; Hamilton, Diamond, Johnson and Ingham 1977; Szeligo and LeBlond 1977; Pappas, Diamond and Johnson 1978; Greer, Diamond and Tang 1981; Cummins, Livesey and Bell 1982; Greer, Diamond and Tang 1982; Katz and Davies 1983; Katz and Davies 1984; Diamond, Johnson, Protti, Ott and Kajisa 1985; Van Gool, Pronker, Mirmiran and Uylings 1987; Diamond, Greer, York, Lewis, Barton and Lin 1987.

[4] The latter finding is not surprising as Altman et al (1968) also reported that the lateral growth of the rat cerebrum comes to a halt sooner than its anteroposterior growth, with an asymptotic level in the width of the

were unable to detect any significant increases in length in rat or gerbil cerebri, following exposure to differential environments for 30 days. This apparent contradiction in findings was resolved by Walsh, Budtz-Olsen, Torok and Cummins (1971) who found that the duration of the experiment was an important factor in eliciting the cerebral effect. These findings have since been confirmed and extended (Walsh, Cummins and Budtz-Olsen 1973) in which study the cerebral component contributing to the increase in cerebral length was found to lie anterior to the region of maximum cerebral width. Temporal characteristics of the development of environmentally induced changes in cortical length have also been explored (Cummins and Livesey 1979) with younger animals demonstrating the least amount of difference. Cerebral length has also been examined in Hooded Lister rats, the strain employed in this thesis (Katz and Davies 1983) and has been found to be affected by behavioural training and testing prior to sacrifice (Crnic 1983). Finally, increasing the complexity of the EC has also been found to have an impact on cortical length, producing greater differences than those found in animals exposed to the more traditional EC (Kuenzle and Knusel 1974), whilst enrichment following impoverishment has also been found to increase cortical length (Katz and Davies 1984).

Subcortical anatomy has also been investigated. Szeligo and LeBlond (1977) have reported that the corpus callosum underlying the occipital cortex was thicker in EC animals and that the number of axons was also greater in this pathway than in the IC animals. The latter finding has been supported by Juraska and Meyer (1986). Of particular interest to researchers, however, have been the studies concerned with the hippocampus because of its known involvement in memory formation (Diamond 1976; Diamond, Johnson, Mizono, Ip, Lee and Wells 1977) and its possible involvement in the behavioural differences between animals reared in complex and isolated environments (Fiala, Joyce and Greenough 1978).

Early work seemed to offer positive results, with for example, Rosenzweig (1966) stating that "preliminary measures indicate that the hippocampus becomes thicker as a consequence of en-

hemispheres being reached at about 20 days that is lateral growth was completed before these animals were exposed to the environments.

riched experience" (p 324). This initial finding was confirmed by Walsh, Budtz-Olsen, Penny and Cummins (1969) who reported a 5.9% difference in the medial area in favour of EC rats and by Rosenzweig (1971) who measured hippocampal thickness in 51 pairs of littermates kept in EC or IC from 25 to 105 days of age. No differences have been reported in hippocampal depth (Diamond, Ingham, Johnson, Bennett and Rosenzweig 1976) or weight (Rosenzweig and Bennett 1978) even under a variety of different environmental conditions (Jones and Smith 1980). However, in Battleboro rats (which have abnormalities in learning and memory) enrichment did have a significant effect on the hippocampus and in micro-anatomical studies, increases have been found in numbers of granule cells in the dentate gyrus of EC animals compared with their IC counterparts (Susser and Wallace 1982), with Walsh and Cummins (1976b) reporting nuclear size in the granule layer being more variable in IC animals.

In recent years, with the increase in sophistication of microscopic procedures, there has been a shift in focus from macro to micro-anatomical analysis of neuroanatomy. In this field too, EC has been found to have a considerable impact on brain morphology. In particular, researchers have considered neuronal and glial density and size, dendritic morphology (number and length of dendrites, counts of the number of spines per unit length of dendrite) and synaptic morphology (number and size).

With respect to neurons, cell counts have revealed a decrease in neuron numbers per unit cortical volume in enriched compared with impoverished animals (Diamond, Krech and Rosenzweig 1964) although there are regional differences in the magnitude of effects (Diamond et al 1964; Cummins and Walsh unpublished data cited in Walsh 1981a). Initially it was assumed that the total number of neurons was fixed (Diamond et al 1964; Rosenzweig 1966) and that the reduction in density in the EC animal was therefore indicative of, and indeed could be used as, an index of cortical expansion. However, subsequent experiments have not supported this index, no significant differences emerging between EC, SC and IC animals in number of neurons per unit measured (Diamond et al 1966; Szeligo and LeBlond 1977). Furthermore recently, Ferchmin

and Eterovic (1986) have provided evidence that cellular multiplication is not a factor in EC-IC effects by showing that the inhibition of putrescine synthesis (resulting in inhibition of cell proliferation) does not reduce EC-IC effects on cortical weight. Therefore, as Walsh (1981a) points out, care must be taken in interpreting the meaning of both neuron density and neuron:glia ratio measures.

Size of neuron has also been examined. Preliminary results (Diamond et al 1966) found no significant differences between the groups in either the size of the perikaryon or the size of the nucleus. However, using a more intense magnification Diamond (1967) did note cell increases in both nuclear and perikaryon size in favour of the EC animal. Largest effects have since been found in periadolescent animals (Malkasian and Diamond 1971) and, as with previous studies, there are regional variations in this effect (Diamond et al 1966; Malkasian and Diamond 1971) and age-duration patterns (Diamond et al 1975).

Measurements of glial cells have also been made, with initial results (Diamond et al 1964; 1966) suggesting that the density of neuroglia is increased in the EC animal. Glial subtypes, however, contribute differentially to this effect with EC-induced increases in oligodendoglia and intermediate cells being primarily responsible for the results. Szeligo and LeBlond (1977) have since confirmed these findings, as well as extending them to include increases in astroglia in EC animals following long (80 days as opposed to the more traditional 30) exposure to their environment. Furthermore, this effect is robust, as Katz and Davies (1984) have shown. In their study no reduction in the EC effect occured even when the animals were subsequently housed in an impoverished environment.

In their original report of increased cortical thickness, Diamond et al (1964) suggested increased dendritic branching as one of the factors contributing to the greater EC cortical bulk. They based this theory on a report (Eayrs and Goodhead 1959) that postnatal cortical development is largely due to dendritic proliferation. It was not for a further two years, however, that the first investigation of the effects of enrichment on dendritic branching was published (Holloway

1966). In this paper individual stellate neurons from the visual cortex were traced using Sholl's (1956) method [5] and EC animals were found to have more dendritic branches than their isolated littermates in 11 out of 15 cases. Although Sholl's method provides an estimate of the total amount of dendritic material and the volumetric extent of the dendritic tree (Greenough 1975) individual dendritic branches and their order of branching have also been examined [6]. Using animals exposed to EC, SC and IC for 30 days Volkmar and Greenough (1972) found that EC animals displayed a greater branching of distal (order 5) branches of basal dendrites in layers II, IV and V pyramidal cells of the visual cortex and layer IV stellate cells. As compared with isolates, standard housed animals showed only small effects in the most distal branches. In a further study (Greenough and Volkmar 1973), order of branching for basal dendrites and oblique branches from apical dendrites were determined independently. EC effects were found mainly in the basal portion (beyond the third and fourth bifurcations) of the pyramidal cell dendritic tree [7]. In a further study (Greenough, Volkmar and Juraska 1973) investigated whether or not these effects were confined to the occipital cortex and found that dendritic branching was also affected in the temporal cortex (layers IV, V pyramidal cells), but not in the frontal cortex. In all the experiments exploring dendritic arborisation reported so far, the environmental experience was started at weaning. Effects have also been found in animals exposed to differential environments when adult, at 112 days (Uylings, Kuypers, Diamond and Veltman 1978), at 145 days (Juraska, Greenough, Elliot, Mack and Berkowitz 1980) and when old, at 600 days (Connor, Melone, Yuen and Diamond 1981) [8]. So enrichment appears to affect the plasticity of cells, although cell types within different regions are differentially affected. As yet the functional significance of these patterns of responding to environmental experience remain to be demonstrated (Renner and

[5] Concentric circles at intervals of 20 microns are drawn around the cell body, the number of intersections the dendrites make with each circle being counted.

[6] Based on a method devised by Coleman and Riesen (1968) order of branching is described as follows: a branch from the cell body is defined as first order, both branches past the bifurcation as second order, both branches after a second bifurcation as third order and so forth.

[7] N.B. Lower order branches are fully developed early in life, hence the main effects occuring in the higher order branches. In addition this study is particularly interesting as effects seem to be prominant in the type of dendrite involved in intercortical communication, pyramidal cells receiving input from recurrent collaterals of nearby pyramidal cells.

[8] For further analyses of dendritic effects please see Connor, Diamond and Johnson (1980); Connor, Diamond, Connor and Johnson (1981); Connor, Beban, Melone, Yuen and Diamond (1982); Connor and Diamond 1982; Connor, Wang and Diamond (1982).

Rosenzweig 1987).

As well as changes in number of dendrites, both dendritic length (Uylings, Kuypers, Diamond and Veltman 1978; Juraska and Greenough 1979; Juraska, Greenough, Elliot, Mack and Berkowitz 1980; Connor, Melone, Yuen and Diamond 1981) and number of spines per unit length of dendrite (Globus, Rosenzweig, Bennett and Diamond 1973; Schapiro and Vukovitch 1970) in the cortex have been found to be altered in favour of the EC animal. Furthermore, as with branching, this increased number of spines is particularly evident in the basal dendrites, which receive primarily intracortical connections. Dendritic effects have also been found in the subcortex, specifically in the hippocampus (Fiala, Joyce and Greenough 1978; Juraska, Fitch, Henderson and Rivers 1985) but in this region the most branching occurs in the inner part of the dendritic tree, and in female animals.

Finally, synaptic morphology has also been explored in the EC/IC literature. The first report of synaptic examination failed to find any consistent differences between EC and IC animals (Bloom 1970). However, that study explored cells from layer I of the cortex, the only layer to be unaltered by enrichment with respect to cortical thickness. Individual synapses in deeper layers of the occipital cortex (layers II to VI) have since been examined with positive results (Mollgaard et al 1971; Diamond, Lindner, Johnson, Bennett and Rosenzweig 1975; West and Greenough 1972; Walsh and Cummins 1976; Bhide and Bedi 1984; Bhide and Bedi 1985; Sirevaag and Greenough 1985; Turner and Greenough 1985). Typically, enrichment as opposed to impoverishment has been found to modify synaptic size, at least as far as post-synaptic thickening of axodendritic synapses is concerned and also to modify synaptic number. In addition, subsynaptic plate perforations (gaps in the post-synaptic thickening) are also affected, higher proportions of occipital cortex axodendritic synapses displaying perforations in EC when compared with IC animals (Greenough, West and DeVoogd 1978). Studies of synaptic contact curvature [9] have also been undertaken with EC rats showing greater concavity than their IC littermates (Wesa, Chang, Greenough and West 1982). Finally Greenough, Hwang and Gorman (1985) have found

[9] A structural feature which, it has been proposed, indicates greater synaptic efficiency (Dyson and Jones 1980).

higher levels of polyribosomal aggregations [10] in the postsynaptic region in EC animals, leading Renner and Rosenzweig (1987) to suggest that "neural activity concomitant with responses to environmental enrichment may actively induce synapse formation" (p 24).

To summarise then, enrichment does seem to have an impact on both cerebral and subcortical weight, cortical depth and hippocampal thickness. In addition, micro-anatomical changes at neuronal level have been found. At this point however, a note of caution should be introduced. Recently the reports of increased depth of cortex in enriched animals have been called into question by Bedi and Bhide (1988) who have highlighted what they consider to be methodological problems in obtaining the samples examined. In addition, they have pointed out that the changes "claimed to have been observed in neuronal perikaryal volumes and nuclear sizes, glial cell numerical densities, synaptic numerical densities and synapse to neuron ratios (are) not obvious and should be regarded with some caution" (p138).

Turning briefly now to the neurochemical effects of differential environments both acetylcholinesterase and cholinesterase respond to the differential environments, displaying a pattern of regional specificity analogous to reported brain weight changes (Zolman and Morimoto 1962; Geller et al 1965; Krech, Rosenzweig and Bennett 1966; Riege and Morimoto 1970; Bennett and Rosenzweig 1968; Rosenzweig, Bennett, Diamond, Wu, Slagle and Saffran 1969; Rosenzweig, Bennett and Diamond 1972; Rosenzweig and Bennett 1978; Bennett 1976; Greenough 1976; Walsh 1980). With respect to the biogenic amines, studies have reported either no significant differences between EC and IC animals (Geller, Yuwiler and Zolman 1965) or small but significant decreases in EC cortical serotonin (Riege and Morimoto 1970). Recently, however, using whole-brain microwave irradiation for sacrifice [11] Renner, Blank, Freeman and Lin (1986) have noted that serotonin turnover rate may be increased in the hippocampus in the IC rat, whilst dopamine is increased in the occipital cortex of EC animals. This work seems to parallel O'Shea, Saari Pappas, Ings and

[10] Location of these aggregates is taken as indication of synapse formation (Renner and Rosenzweig 1987).

[11] This technique, which Renner and Rosenzweig (1987) advocate, reduces the continued enzymatic activity that typically occurs post-mortem in the more usual methods of sacrifice, thus making the sample more representative of the enzyme activity occuring in the live organism.

Stange's (1983) discovery that isolation rearing decreased dopamine levels of the hypothalamus and posterior cortex. Both norepinephrine concentration and total brain norepinephrine have also been examined and have been found to be significantly higher in IC animals (Geller et al 1965) these differences manifesting themselves in the caudate nucleus (Geller and Yuwiler 1968; Geller 1971). More recently, Pappas, Saari, Smythe, Murtha, Stange and Ings (1987) have suggested that forebrain norepinephrine "is permissive to the deleterious behavioural consequences of restricted experience during maturation" (p153).

As with the neurotransmitters, both RNA and DNA content have also been investigated in animals housed in differential environments, and it is now clear that total DNA is largely unaffected by environmental complexity (Rosenzweig et al 1972; Ferchmin and Eterovic 1986) but environmental enrichment does appear to affect both RNA and RNA/DNA ratios (Rosenzweig and Bennett 1977; 1978; Rosenzweig et al 1972; Ferchmin et al 1970; Ferchmin and Eterovic 1986; Essman 1971). Qualitative changes in gene activation and RNA diversity have, however, been more difficult to determine. It seem likely that the multitude of morphological and metabolic effects which follow differential rearing are at least partially due to differential activation of the genome so that transcriptional activity of the genome to effect RNA synthesis is modified (Walsh 1980; Uphouse and Bonner 1975; Grouse, Schrier, Bennett, Rosenzweig and Nelson 1978; Uphouse and Moore 1978; Uphouse 1978; Uphouse and Tedeschi 1979).

Finally, there has been some investigation of protein changes following exposure to differential environments (Bennett et al 1964; Krech et al 1966; Das and Altman 1966; Levitan et al 1972a; 1972b; Welch, Brown, Welch and Lin 1974; Jorgensen and Meier 1979; Jorgensen and Bock 1979; Hyden and Ronnback 1979). To date, however, little is known of the precise relationship of these biochemical responses to the neural electrical activity which induced them or to the final long term anatomical and chemical effects which they mediate (Walsh 1981b) although Leah, Allardyce and Cummins (1985) have reported that evoked cortical potentials from enriched rats showed a decreased amplitude with somatic stimuli whereas those from isolated animals did

not. This has led them to suggest that "isolation impairs development of control of sensory input and that this is reflected in somatosensory cortical electrical and behavioural responses to environmental stimuli" (p 27).

From the above several interesting points emerge. Firstly, and most importantly, mere exposure to environmental experience has an impact on brain morphology and chemistry. Furthermore, in most cases, animals exposed to environmental enrichment appear to undergo anatomical changes of a beneficial nature, when compared to their impoverished counterparts. Despite the criticisms of the methodologies employed (Bedi and Bhide 1988), morphological changes in the anatomical measures taken are consistent across age, sex and duration of exposure to environments and remarkably robust. Finally, when considering all the evidence, the pattern that emerges suggests that active interaction with the environment has a positive impact on a variety of increasingly microscopic measures.

Despite the large literature on the effects of differential experience on brain and behaviour, however, the *causes* of these differences are less clear. To date, there are few environmental characteristics, or even subject characteristics, that have not been singled out at on time or another and proposed to be the critical difference between EC and IC animals. The earliest reports of neural plasticity induced by differential environments included control experiments to examine the possible roles played by handling and locomotion in these effects (Krech, Rosenzweig and Bennett 1960). Since then a variety of factors has been suggested as causing the EC/IC differences (Rosenzweig and Bennett 1976; Renner and Rosenzweig 1987), including different rates of maturation (Cummins, Livesey, Evans and Walsh 1977) stress (Geller 1971) arousal (Walsh and Cummins 1975) as well as endocrine system alteration (Rosenzweig and Bennett 1984; Renner 1987) differential motivation (Lamden and Rose 1979; Chadha and Rose 1981; Rose et al 1986; 1987) and social forms of play (Einon, Morgan and Kibbler 1978; Einon, Humphreys, Chivers, Field and Naylor 1981). A recurring hypothesis in the literature, however, is that the differences observed between EC and IC animals are the result of learning in the enriched

environment that does not occur in the impoverished environment (Rosenzweig and Bennett 1976). This idea is a direct descendant of the hypothesis that led to the first work with differential environments at Berkeley and is perhaps the most intuitively satisfying of all the explanations that have been advanced. However, it is difficult to obtain direct experimental evidence supporting or refuting the hypothesis that learning is responsible for the EC/IC differences, the hypothesis typically only gaining support from indirect sources, for example with the rejection of alternative explanations. In general, much of the research directed towards elucidating causes of EC/IC effects has focussed on single variables. However, as Renner and Rosenzweig (1987) point out, it would be overly simplistic to assert that one or the other variable is *the* cause of these differences and it is probable that the neural and behavioural responses to environmental manipulation are the "cumulative result of compound synergistic influences of several different types of variables" (p 90). For an in depth analysis of the possible causes of the enriched effect, the reader is refered to Renner and Rosenzweig's (1987) review.

Today the study of the effects of differential environments in a wide range of species including various strains of *rats* (Krech, Rosenzweig and Bennett 1960; Ferchmin, Eterovic and Levin 1980; Greer, Diamond and Murphy 1982), *mice* (LaTorre 1968; Henderson 1970; 1973; Cummins, Livesey and Bell 1982; 1983), *gerbils* (Rosenzweig and Bennett 1969; Cheal, Foley and Kastenbaum 1984; 1986), *guinea-pigs* (Sahakian and Robbins 1975) *ground squirrels* (Rosenzweig, Bennett and Sherman 1980; Rosenzweig, Bennett, Alberti, Morimoto and Renner 1982; Rosenzweig, Bennett, Renner and Alberti 1987), *Peking ducks* (Heaton and Klein 1981), *chicks* Jones (1982), *cats* (Cornwell and Overman 1981; Wilson, Warren and Abbott 1965) and *monkeys* (Gluck, Harlow and Schiltz 1973) constitutes an important and considerable area of research which is of potential value in a number of contexts. These will be outlined in the following section.

1:5 IMPLICATIONS OF ENRICHMENT EFFECTS

Apart from the obvious implications of environmentally induced brain changes for the nature versus nurture debate, this paradigm offers a method of studying the relationship between brain and behaviour. The initial hypothesis of the Berkeley group was that individual differences in brain chemistry might be related to individual differences in learning ability. Their research did yield significant results, (Rosenzweig, Krech and Bennett 1958) and later they found significant correlations between brain weight measures and learning in several strains of rats (Rosenzweig, Bennett and Diamond 1967). Since this early work, it has become increasingly clear that the plasticity of the nervous system plays a major role in the storage and processing of information and it has been through the studies of the effects of differential environmental experience that much of the detailed information pertaining to brain plasticity has emerged (Will, Schmitt and Dalrymple-Alford 1985; Renner and Rosenzweig 1987). In particular, studies of enrichment have identified various areas of neuroanatomy and neurochemical function that are particularly susceptible to changes in the external environment, thus highlighting areas which warrant more detailed investigation (Greenough 1976). In addition, this documentation of the various areas sensitive to change and their differences in responsiveness have afforded clues about the localisation of neural functions and thus to the relationship between brain and behaviour, without having to resort to invasive techniques. As noted earlier this area of research has obvious applications for our understanding of higher order processing, without having to work with lesioned animals.

In addition, the findings of brain plasticity in adult and even in geriatric rats (Connor, Melone, Yuen and Diamond 1981; Cummins, Walsh, Budtz-Olsen, Konstantinos and Horsfall 1973; Kubanis, Zornetzer and Freund 1982) has challenged the assumption of many psychologists and neuroscientists that the brain assumes adult values early in life (Rosenzweig 1984). Apparently these findings have influenced the thinking of a number of developmental psychologists, for example, in calling for more intensive studies of the memory of older persons (Honzik 1984) and of how best to help to keep people fit in advancing age (Sandman and Donnelly 1983).

The use of enriched experience to promote recovery of function after brain damage and the mechanisms of this effect have also been a topic of investigation. Schwartz (1964), for example, has shown that after cortical lesions had been inflicted on neonatal rats, subsequent housing in an enriched environment led to better maze performance than was found in lesioned rats housed under standard colony conditions. The beneficial effects of post-lesion environment have not only been found in neonatal animals (Will, Rosenzweig and Bennett 1976), however, but also in post-weaning (Will, Rosenzweig, Bennett, Hebert and Morimoto 1977) and in adult animals. (Will and Rosenzweig 1976). In addition, environmental enrichment has been found to partially ameliorate the effects of undernutrition, although the details of these benefits are still subject to disagreement (Levitsky and Barnes 1972; Sara, King and Lazarus 1976; Katz and Davies 1983; Bhide and Bedi 1982; 1984). Elements of enrichment employed as a therapeutic environment can be found in a variety of human situations too, for example in fostering the development of retarded children (Hayden and Haring 1984) and in the use of conductive education at the Pétö Institute in Hungary (Hari and Akos 1988; Hari 1989) and more recently at the Birmingham Institute for Conductive Education. The work with deaf-blind children at the Moscow Institute in Russia (Lambert 1987; 1988) and Feuerstein's work improving the social and cultural abilities of maladapted children in Israel using "instrumental learning" (Sharron 1987) can also be seen in this context. Ideas about recovery of function have evolved rapidly in recent years, with demonstrations that the brain and spinal cord are not static organs. Stimulation and training aids have been increasingly investigated and some recent volumes include papers devoted to this topic (Almli and Finger 1984; Bach y Rita 1980; Van Hof and Mohn 1981). Enrichment has been found to aid compensation for lost function when employed as a post-lesion therapy (Rose et al 1988) and to protect against the deleterious behavioural deficits when employed as a pre-lesion preventative measure (Dalrymple-Alford and Kelche 1985). Obviously this work has furthered our knowledge about the mechanisms subserving recovery of function after brain damage. For a more detailed discussion, the reader is referred to Rose (1988) and to Will and Kelche (forthcoming [12]).

[12] In Rose F.D. and Johnson D.A. (Eds) Recovery of function following brain damage. London: Plenum Publ.

Animals, too, are benefitting from the EC/IC findings. Bennett and Rosenzweig (1981) have advocated enriching the environments of laboratory animals, so that they are more representative of their species. In addition some of the attempts to provide zoo animals with more natural and more complex environments draw upon the Berkeley group's laboratory work (White 1975; Markowitz 1982; Markowitz and Spinelli 1986). In the case of farm animals, research is showing that an enriched environment is helpful in raising pigs and calves. The animals grow better and are less aggressive when raised in moderately complex environments rather than in barren conditions (Woodgush, Stolba and Miller 1983). The growing interest in the latter application is reflected in the increasing number of publications, for example in Applied Animal Ethology, and The International Journal For The Study Of Animal Problems (Rosenzweig 1984). Improving the quality of livestock has tremendous economic implications and must be considered as one of the most important practical applications of this area of research.

To summarise, attempts to improve health and behaviour by providing enriched environments are now being seen in many situations, ranging from the housing of laboratory, zoo and farm animals, to encouraging normal development in retarded children, improving the quality of life in the elderly, and promoting recovery after brain damage or malnutrition. All of these applications concern organisms *directly* exposed to the enriched environment.

A recent and exciting extension of this area, however, concerns the effects of differential environments on *offspring* behaviour. Within this field, a handful of studies already exist that have found significant anatomical and behavioural differences in progeny of enriched compared with impoverished animals and it is this area of research which provides the point of departure for this present thesis, the historical background of which will be described below.

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1:6 TRANSFER OF EFFECTS ACROSS GENERATIONS

Over the last few years there has been a growing interest in the effects of differential maternal experiences on their offspring. The notion that the environment can influence future generations is not new. Indeed in the Orient and particularily in Japan, there is a longheld belief that the cultural enrichment of a pregnant woman can influence the intelligence of her unborn child. This intrauterine education is known as *Taikyo*, and can be traced back more than two thousand years in Chinese literature (Nakae 1983). Despite this tradition, however, it is only comparatively recently that experimental studies have been reported which appear to corroborate this popular concept (Kiyono et al 1985).

Within the EC/IC literature, there are several studies in which female rats and their litters have been reared in enriched environments postpartum, and a few experiments where the research procedure has incorporated some prenatal environmental experience too. Methodologically, in these studies which are outlined in Table 1:1, it is almost impossible to separate out the maternal influence from the offsprings' *direct* experience of enrichment, as both mothers and offspring are exposed to enrichment. However, these studies do have something in common with the present thesis, namely the effects of enrichment of the offspring could in some way be mediated by their interaction with their mother and as such merit a brief mention in this introduction.

Of *direct* relevance to the present research are those few studies in which the effects of enrichment of the parent generation on their offspring have been the *focus* of the research, and where procedures such as cross fostering the offspring generation postpartum, or confining the differential environmental experience to the parent generation alone have allowed the transfer of the effects to the offspring to be studied in some detail. This research is central to the present thesis and as there are only a few studies, the relevant work in this field will be reviewed in the following pages, rather than in a separate chapter.

AUTHOR	EXPERIENCE	EXPERIMENTAL TESTS	FINDINGS
Forgus 1956	Perinatal	Visual Discrimination	Early Experience Superior to Late Experience
Dawson and Hoffman 1958	Perinatal Postnatal	Open Field Hebb Williams	EC>SC (Exploration) EC>IC
Forgays and Read 1962	Perinatal	Y Maze Hebb Williams	EC>SC EC>IC
Schwartz 1964	Perinatal Postnatal	Hebb Williams	SC>EC Lesioned EC>Others
Whimbey and Denenberg 1966	Prenatal and Perinatal	Wide Battery of Tests Employed	Effects of Mothers Found on Offspring
Ravizza and Herschberger 1966	Prenatal Perinatal	Table Top Exploration Hebb Williams Activity Wheel	EC>SC EC>SC EC>SC
Whimbey and Denenberg 1967a	Prenatal Perinatal	Wide Battery of Tests Employed	Results Factor Analysed
Whimbey and Denenberg 1967b	Perinatal Postnatal	Wide Battery of Tests Employed	Mainly Reporting Open Field Reliability
Denenberg, Woodcock and Rosenberg 1968	Prenatal Perinatal	Hebb Williams	SC>EC (No. of Errors)
Denenberg and Whimbey 1968	Prenatal Perinatal Postnatal	Wide Battery of Tests	Some Pre-Postnatal Interactions
Denenberg 1969a	Perinatal Postnatal	Open Field Avoidance Conditioning Consummatory Behaviour	Included Handling Emotionality/Activity Differences
Manosevitz 1970 (Mice)	Perinatal Postnatal	Open Field Running Wheel Food Competition	EC>SC EC>SC EC>SC
Konrad and Bagshaw 1970 (Kittens)	Perinatal Postnatal	Novel Room With Objects	Differences in Rates of Developmentof Play Sequences in Favour of EC

Table 1:1 Brief review of all the studies that have employed differential environments prenatally, perinatally (that is before weaning), or postnatally (after weaning), but have not controlled for the *direct* effects of environmental experience on the offspring.

Key: EC Enriched Condition; IC Impoverished Condition; SC Standard or Control Condition; > and < indicate direction of effects.

Malkasian and Diamond 1971	Perinatal Postnatal	Docility Body Weight Body Length Testicular Weight Cortical Depth Nucleic Perikarya	SC>IC IC>SC (at 28 days) IC>EC (at 14 days) EC>IC (at 14 days) EC>IC (at 14 days) EC>IC (at 19 and 28 days) IC>EC
Smith 1972	Perinatal Postnatal	Open Field Hebb Williams	IC>EC Significant Environmental Effects
Manosevitz and Montemayor 1972 (Mice)	Prenatal Perinatal Postnatal	Open Field Exploration Running Wheel	EC>SC EC Habituated Faster SC>EC
Henderson 1972 (Mice)	Perinatal Postnatal	Brain Weights Body Weight	EC>SC EC>SC
Manosevitz and Joel 1973 (Mice)	Prenatal Perinatal Postnatal	Open Field Open Field Running Wheel Exploration Hoarding Body Weight Adrenal Weight	Activity EC>SC Defecation SC>EC EC>SC EC>SC EC>SC EC>SC EC>SC
Manosevitz and Pryor 1975 (Mice)	Prenatal Perinatal Postnatal	Body Weight Open Field Open Field Water Consumption	EC>SC Activity EC>SC Defecation SC>EC EC>SC
Sjoden and Soderberg 1975	Prenatal Perinatal	Open Field	SC>EC
Will, Rosenzweig and Bennett 1976	Perinatal Postnatal	Hebb Williams Cerebral Width	Environmental Effects (no details)
Henderson 1977 (Mice)	Perinatal	Climbing	EC>other groups
Ivinskis and Homewood 1980	Prenatal Perinatal	Hebb Williams Hebb Williams	N/S EC>SC
Nau, Elias and Bell 1981	Perinatal Postnatal	Bar Pressing	SC>EC
Jones 1982 (Chicks)	0-7 Days	Novel Environment Latency to Emerge	Immobility SC>EC SC>EC
Muir, Pfister and Ivinskis 1985	Perinatal	Hebb Williams	Errors SC/IC>EC
Venable et al 1988	Perinatal Postnatal	Hebb Williams	No of Errors SC>EC Latency to Leave Start Box SC>EC Running Time SC>EC

Table 1:1 continued.

The first work to consider the intergenerational effects of environmental experience emerged from a series of studies in the 1950's and 1960's, by Victor Denenberg and his colleagues. In an attempt to understand the adult organism's behaviour and having established that a variety of experiences have an impact on subsequent performance (Denenberg 1970) they combined these various experiences into a "spatial and temporal" experimental framework. This allowed them to programme life histories, and produce heterogenous subgroups with marked behavioural differences, which Denenberg terms "experimentally produced individual differences or personalities" (Denenberg 1970 p63). Within this framework, one manipulation which falls within the class of events best described as "social interactions" (Denenberg 1969a p22) was the exposure of the mothers of the experimental subjects to differential environments (Denenberg and Rosenberg 1967), in particular, preweaning housing of future mothers in either stainless steel maternity cages, or in free environment boxes, and postweaning housing of future mothers in a stainless steel laboratory cage, or in a free environment. In addition, half of the animals were offspring of females which had been handled preweaning. The experimental subjects, offspring of the animals exposed to the differential environments, were given one three-minute open field test and weighed. The data for activity levels and weaning weights revealed firstly, that the nature of the mother's living quarters during her early life will affect her offspring and secondly, that handling females (the grandmothers of the experimental subjects) in infancy can have an effect two generations futher on. Examination of the means tabled in the 1967 paper shows that postweaning housing of mothers in a free environment produced offspring that were more active but less heavy than offspring of mothers housed in laboratory cages.

Direct investigation of the effects of differential maternal environments on offspring, without involving additional variables such as handling in the design, was first reported in 1971 when Diamond, Johnson and Ingham published a paper entitled "Brain plasticity induced by environment and pregnancy". This paper included a report on the offspring of parents experiencing varied environmental conditions prenatally. In this instance, Diamond et al discovered no significant differences in pups from EC vs IC parents in number of offspring, number of implantation

sites, or in cortical depth measures. However, they did find that the pups from the EC parents had significantly greater birth weights than pups from IC parents. This birth weight difference is interesting, as it is in the opposite direction to the weaning weight differences observed by Denenberg and Rosenberg (1967). The lack of significant differences in the offspring brain morphology present somewhat of a paradox, namely no apparent offspring differences in cortical depth measurements (Diamond, Johnson and Ingham 1971) yet differences in Open Field behavior (Denenberg and Rosenberg 1967). This might, however, be explained in two ways. Firstly, it is possible that morphological differences did occur in the offspring brain, but that they were at an ultrastructural level (Diamond et al 1971) and therefore were not detectable by cortical depth measurement. Secondly, that for any prenatally mediated brain changes in the pups to manifest themselves, perhaps a degree of postnatal interaction with the mother or siblings is required (Ivinskis and Homewood 1980). In Diamond et al's study, all the animals were sacrificed immediately after birth. Perhaps a delay prior to sacrifice, of either a few days, or even until the animals were weaned would have produced significant brain changes? More recently, in "Psychology Today", an interview with Marion Diamond (NOV 1984) gave a clue to understanding this apparent paradox, which fits in with the explanations offered above.

> "... we found that rat pups from the enriched parents have increased body weights at birth, but the cortex does not show significant change. Then we wondered if we would see cortical differences when these pups grew up. And we did ! ..." (Diamond 1984 p 68)

Housing of these animals was in a standard cage, that is, with no direct enrichment, but their brains were still larger as adults. In fact Diamond reports that

> " ... We're up to the third generation and the brains are still enlarged ..." (Diamond 1984 p 68)

yet her explanation for the findings is quite simply that the pups coming from the enriched

parents have greater body weights, reflecting the fact that brain weight is known to vary with body weight (Rosenzweig and Bennett 1978). The findings of differences between the offspring of animals directly exposed to the differential environments have been reported in slightly more detail elsewhere (Diamond, Chui, Johnson, Chelgren, Greer and Gibbons 1984) in which abstract, cortical thickness differences between male progeny occipital cortices were described. Data is being analysed for the frontal, parietal and occipital cortices from male and female rat progeny, across three generations, (Diamond Jan 1986 personal communication), which will reveal a clearer picture of the anatomical effects [13].

As well as anatomical investigations, the behavioural consequences of prenatal maternal enrichment and restriction on offspring have also been examined. One of the earliest studies was that of McKim and Thompson (1975) who exposed female rats during pregnancy to enriched, restricted or normal cage environments and at parturition cross-fostered whole litters from each biological mother to a foster mother that had occupied a prenatal environmental condition either different from or the same as that occupied by their biological mothers, thus generating a nine cell design representing all possible combinations of pre and postnatal treatments. This elegantly designed experiment revealed that variation in environmental complexity undergone by female rats during pregnancy can produce definite changes in offspring behaviour, specifically offspring open field performance. In particular, offspring of enriched animals reared by an enriched foster mother were more active than offspring of control or restricted mothers, reminiscent of the earlier findings of Denenberg and Rosenberg (1967).

Probably the most interesting findings on the behavioural consequences of prenatal maternal environment on offspring performance as they involve offspring learning abilities, have been published by a group of Japanese researchers led by Dr. S. Kiyono. In an early paper (Kiyono, Seo and Shibagaki 1982) they reported that facilitative effects of prenatal environmental enrichment in rats could be observed as a decrease of initial error scores in Hebb-Williams maze learning in the offspring, as compared with environmentally impoverished mothers' offspring. In 1985,

[13] At the time of submission of this thesis, this data was still not published.

Kiyono, Seo, Shibagaki and Inouye extended these early findings, as their initial sample was too small to form any definite conclusions. In addition, in the later study, they included a standard colony condition (SC) as a control reference, as well as a cross fostering element in which half the male progeny were reared by their biological mothers and half by foster mothers. This rather complex design is summarised in Table 1:2 and was instigated to examine the contribution made by the prenatal and postnatal mothers separately. At 21-25 days, subjects were weaned and housed according to rearing conditions and then trained and tested on the Hebb-Williams maze. After completion of this experiment, animals were sacrificed and brain samples taken. The results of this research are most interesting and will be described in detail below.

PRENATAL MATERNAL EXPERIENCE	POSTNATAL MATERNAL EXPERIENCE	NATURAL OR FOSTER MOTHER
Enriched	Enriched	Natural
Standard	Standard	Natural
Impoverished	Impoverished	Natural
Enriched	Standard	Foster
Standard	Standard	Foster
Impoverished	Standard	Foster

Table 1:2 Diagramatic representation of the breeding design employed by Kiyono et al (1985).

Firstly, they found no significant differences between the groups with respect to both littersize and birth weights. The latter measure is of particular interest as in this instance IC progeny tended to be heavier than EC progeny in direct opposition to the finding of Diamond et al (1971). Secondly, total errors in the Hebb-Williams maze over the 12 test problems revealed a significant difference between offspring of EC and IC mothers, but not between offspring of EC compared with SC mothers or offspring of SC compared with IC mothers. Interestingly, no significant effects were found due to fostering, nor was there an environment by fostering interaction. The latter point was futher examined in a second experiment in this study, which will be described below. Thirdly, after completion of the experiment, the rats were sacrificed and

wet brain and cerebral weights were measured, as was thickness of the occipital cortex. Dendritic spines on the pyramidal neurones were also counted. Curiously, neither the brain weights nor the cortical thickness yielded significant effects of environment or fostering, although Kiyono et al report that the values of the EC offspring were larger than those of the IC offspring, with offspring of SC females situated between the EC and IC groups, for all measures. The number of dendritic spines also revealed no specific differences between the groups. Overall, therefore, although no significant differences emerged, unlike those reported by Diamond et al (1984) on reflection, this is not unusual, as both the IC and SC groups had undergone intensive training and testing on the Hebb-Williams maze prior to sacrifice, which would have altered their brain anatomy (Rosenzweig and Bennett 1977) .

In the second experiment, which was conducted to extend the initial findings, pregnant EC dams were allowed to explore a Hebb-Williams apparatus three times a week as well as being maintained in their enriched environment. In addition, all litters were cross fostered to SC dams. Interestingly, in this experiment the body weights of the offspring of EC mothers were heavier than the offspring of SC mothers at the end of testing, with no differences being found between the progeny of SC and IC dams. Typically, with animals directly exposed to the EC/IC environments, the opposite effect occurs, namely IC animals weigh more than EC animals (Fiala Snow and Greenough 1977). The total error scores for the three groups were generally similar to the the data observed in the previous experiment, with progeny of EC dams making fewer errors than the progeny of SC and IC dams.

In summary, the possible effects of postnatal maternal influences were examined in experiment one in which the offspring were reared either by their own mothers or by foster mothers and in experiment two in which all offspring were reared by foster mothers. Kiyono et al conclude that the design allowed the postnatal maternal contribution to offspring behaviour to be eliminated and propose that the results can be attributed to prenatal influences. Secondly, they conclude that prenatal maternal enrichment aids offspring learning in the Hebb-Williams maze and thirdly

that post-testing, no significant brain differences exist in the progeny.

Finally, in their most recent publication, Inouye Kiyono Seo and Shibagaki (1986) have re-examined the effects of prenatal enrichment on offspring brain morphology. Dams were exposed to EC, SC or IC during gestation, being re-housed in standard colony cages on day 20 of gestation, just prior to parturition. Offspring were reared by their biological mothers until weaning and then kept in SC (N=4 per cage) until 32 days old, at which point they were sacrificed. Brain wet weights were recorded and dendritic spine counts taken from Kreig's areas 17, 18a and 39. Results indicated that there was a non significant tendency for the EC brain to be heavier than its IC or SC counterparts', reminiscent of Diamond et al's (1984) findings. Moreover, there was also a significant increase in the number of dendritic spines in the EC offspring when compared with the other two groups, a finding in opposition to their earlier work (Kiyono et al 1985).

To summarise the effects of differential maternal environments on their offspring, considering all the studies reported above, several striking facts have emerged. Firstly, physiological and neuroanatomical differences have been found between the progeny. If examined directly post-partum, no brain differences exist with respect to cortical thickness, (Diamond, Johnson and Ingham 1971) but allowing the animals to mature does produce significant differences in occipital cortex thickness in favour of the enriched progeny (Diamond et al 1984). However, these differences disappear in a post-testing situation (Kiyono et al 1985). Similarly, there are no cerebral weight or dendritic spine count differences between the groups, after training and testing in the Hebb-Williams maze (Kiyono et al 1985), although dendritic spine count differences have been found in untrained animals (Inouye et al 1986). Animal body weights are also affected by maternal environment. At birth, offspring of EC dams weigh more than offspring of IC dams (Diamond, Johnson and Ingham 1971) although Kiyono et al (1985) were not able to replicate this. Weaning weights seem to show the opposite pattern, with progeny of IC mothers reported as heavier than progeny of EC mothers in the Denenberg and Rosenberg paper (1967). However, post-testing, Kiyono et al (1985) found the original EC/IC difference re-emerging. In none of the

papers was there any differences in littersize, number of offspring or number of implantation sites reported. With respect to the behavioural measures, both activity levels and learning seem to be influenced by the maternal environment. Both Denenberg and Rosenberg (1967) and McKim and Thompson (1975) found EC offspring to be more active than IC offspring, as measured in an Open Field paradigm. Moreover, in both experiments reported by Kiyono et al (1985) EC offspring made fewer errors than their IC and SC counterparts.

As would be expected with such a new area of research, few causes of these effects in the offspring have been proferred to date. With respect to the anatomical differences, Diamond (1984) has suggested that the increased cortical dimensions in the offspring of enriched dams might simply reflect the observed increases in body weight in these animals. However, more recently, in the Brain and Mind Bulletin (March 1987, Vol 12, Number 7) reporting on a paper that Marion Diamond gave to the annual conference for the Gifted in Los Angeles, another solution was suggested. Diamond, according to the article, has speculated that the mechanism for the transfer of effects across generations might be mediated in part by progesterone which can cross the blood-brain barrier. This fits in with Kiyono et al's (1985) suggestion that the "maternal biochemical changes produced by enrichment may have altered the intrauterine environment of the fetuses" (p434), thus mediating the changes in Hebb-Williams performance that they observed. Other than these physiological explanations, however, one other which has intuitive appeal, has been suggested by Ivinskis and Homewood (1980) who implicate maternal behaviour as a mechanism for transferring experience. In particular, they suggest that when mothers (and in their experiments, the pups) are exposed to an enriched environment, the effects of this early experience are mediated to the pups by a higher internal arousal caused by extra stimulation from their mothers.

Whatever the causes, the research outlined above clearly demonstrates that the effects of differential environmental experience can be transferred across generations and provides the focus of the present thesis, the details of which will be outlined in the next section.

1:7 PURPOSE OF PRESENT THESIS

In this introduction, several important themes have emerged which drawn together provide the roots of the present work. Historically, there has been a continuing and often philosophical debate about the *nature* of mankind with the relative contributions of heredity and environment to the development of any individual continually being reassessed. Contemporary psychologists are of the opinion that both biological predispositions and environmental forces interact to produce the complexity of human attributes (Shaffer 1985), with methodological interest focussed increasingly on how the environmental components of the equation interact with each other (Anastasi 1958; Denenberg 1982). This is particularly obvious when the animal literature, from which most of the experiential research has emerged, is considered. Typically both the timing and the nature of the experience has been manipulated, with both beneficial and deleterious results.

The present thesis, taking the view that the ameliorating effects of the experience is of primary importance, has focussed on one literature in particular, namely differential environmental experience. Exposure of animals to environmental enrichment has been found to have beneficial neuroanatomical, neurochemical and behavioural consequences, with implications for the quality of life in both animals and humans (Rosenzweig 1984; Renner and Rosenzweig 1987). Within this literature are a few studies which indicate that these beneficial effects might not only be confined to those animals directly exposed to the experience of an enriching environment, but might be transferred across generations. The impact of intergenerational effects has of course been researched using manipulations other than enrichment and has a long history in the orient (Nakae 1983). Indeed, it has long been established that various kinds of stressors imposed upon females of different species can affect both the physiology and behaviour of their offspring. (Reviews eg: Joffe 1969a; 1969b; Thompson and Grusec 1970; Archer and Blackman 1971; Joffe 1978; 1982). Within this field both prenatal, that is during pregnancy, and pre-pregnancy paradigms have been employed. In addition the nature of the stressors have been both "physical" in which procedures are employed which appear to be physically stressful (eg Handling - Ader and Con-

klin 1963; Ader and Plaut 1968) or involve a painful component (eg Footshock - Joffe 1977) or "psychological", which include procedures that are neither physical nor painful. However, few of these manipulations can be seen as necessarily ameliorating, hence the decision in the present thesis to concentrate on the intergenerational effects of environmental enrichment rather than some of the other methodologies that are currently available.

To date few studies have explored the effects of environmental enrichment on successive generations, although there is now evidence that both physiological and anatomical differences have emerged between the progeny of differentially housed mothers. The present thesis was therefore designed to complement and extend this work by compiling a more complete *behavioural* profile of offspring of differentially housed mothers. In addition, the behavioural investigation in the current work was extended across three generations of animals, namely animals directly exposed to differential environments, their offspring and their grandoffspring.

Finally, although manipulation of the environment of the parent generation is not a new experimental phenomenon, other than the paper reported by Denenberg and Rosenberg (1967), the handful of studies examining the behavioural effects of differential environments on offspring, all employed a prenatal paradigm, that is the manipulation occured during pregnancy. Employing an experimental procedure during pregnancy, however, does present a dilemma. Procedures may affect the foetus directly (Joffe 1978), rather than being mediated by the mother and would thus be comparable to exposing the animal directly to the differential environments. In the present research, this factor was taken into account, by employing a paradigm similar to that employed by Denenberg and Rosenberg in their innovative 1967 paper, namely, a pre-pregnancy paradigm [14]. This was to ensure that any effect found in the offspring could only have been mediated by the mother.

[14] A review of the main findings of the effects of manipulating the maternal generation on offspring behaviour, using paradigms other than differential environments will be presented in chapter three of this thesis.

1:8 EXPERIMENTAL PROGRAMME OF PRESENT THESIS

As outlined above, the general purpose of this thesis was to investigate the effects of differential maternal environments, prior to pregnancy, on future offspring. In particular, the experimental programme was designed to examine three areas of interest, which are outlined below.

- Firstly, this thesis aimed to provide a behavioural profile of animals raised in the differential environments, both to validate the use of the superenriched environment employed in this thesis and to serve as a baseline against which the behavioural profiles of the offspring and grandoffspring could be compared. Study one (chapter five) therefore consisted of two experiments: the first designed to establish a behavioural profile of male and female rats directly exposed to the differential environments, using measures of activity and emotionality (open field), perception (visual cliff) and learning (Skinner box) and the second to check that the environmental effects continued postpartum in the females.

- Secondly, this thesis was designed to establish a behavioural profile of the offspring of differentially housed mothers. Study two (chapter six) investigated whether or not behavioural differences existed in the offspring of differentially housed mothers using the same battery of tests as was employed in the previous study, to allow a comparison of behavioural profiles of offspring and parental generations to be made. In addition, the possibility of effects being transferred over two generations were also examined in a second experiment in this study.

- Finally, a further investigation of the *nature* of the behavioural differences observed in the offspring was conducted. Of particular interest was whether there was a learning difference per se, or whether differential performances in the operant conditioning paradigm of study two merely reflected an activity difference. Study three (chapter seven) consequently employed a maze learning task and a Skinner box conditioning task which has previously been found to equate motivational differences in EC/IC animals (Rose, Love and Dell 1986). To further investigate the learning versus activity question, study four (chapter eight) artifi-

cially manipulated activity levels using amphetamines as advocated by Walsh and Cummins (1975).

In addition to these four experimental chapters (chapters five to eight inclusive) and this present introduction, this thesis also contains two review chapters, a methodology chapter, and a final discussion chapter. More specifically, chapter two reviews the behavioural characteristics of animals exposed *directly* to differential environments, including the paradigms employed in this present work, against which to compare the offspring findings from the present research. The effects on offspring of manipulations other than enrichment imposed upon the maternal generation prenatally, are the subject of chapter three, whilst the methodologies employed in the present experiments and the analyses used will be described in detail in chapter four. A résumé of the findings and consideration of the wider implications of this research will be provided in the final discussion in chapter nine.

CHAPTER TWO:

OVERVIEW OF THE LITERATURE INVESTIGATING THE EFFECTS OF DIRECT EXPOSURE TO DIFFERENTIAL ENVIRONMENTS ON BEHAVIOUR

2:1 INTRODUCTION

The first investigation of the effects of maternal environments on offspring revealed a *behavioural* difference between the progeny of enriched and impoverished dams (Denenberg and Rosenberg 1967). Since this early study it has become apparent that both offspring activity levels and performance in learning tasks are affected by differential maternal experience (McKim and Thompson 1975; Kiyono et al 1982, 1985). Behavioural effects associated with *direct* exposure to environmental enrichment have also been well documented. The purpose of the present review is to outline the main findings in the latter area to provide a profile against which to set the behavioural effects in *offspring* of differentially reared animals examined in the present work. Furthermore, as the complexity of the effects of differential environments on behaviour only become apparent when the full profile of enriched and impoverished animals is considered, the present review aims to delineate this more complete picture, rather than just focussing on those experimental paradigms employed in this thesis.

"Just as manipulation of the complexity of the environment in which an animal is raised leads to changes in the brain, experimental manipulation of the environment has a measurable impact on behaviour" (Renner and Rosenzweig 1987 p 39). In fact, this issue was studied prior to the search for neural correlates of differential experience (see chapter one). As early as 1947, Hebb reported behavioural differences between rats reared at home as pets and rats reared in laboratory cages. Since then, the majority of investigations of behaviour in differentially housed animals have focussed on direct measures of learning and memory. Implicit in this avenue of research is the notion that animals with an enriched experience and with the concomitant increases in brain measures when compared with their impoverished counterparts, will also be behaviourally superior. As Renner and Rosenzweig (1987) point out, however, "this inference often goes unstated and even unexamined". Furthermore, "although animals housed in complex environments are different from those housed in impoverished environments, the linkages between the brain changes and alterations in behaviour are not obvious" (p40).

Despite this cautionary viewpoint, over the past four decades a large literature examining the behavioural characteristics of differentially reared animals has emerged, which will be outlined in this chapter. As well as investigating the effects of EC and IC on learning and memory, other facets of behaviour have also been studied, including activity levels, perceptual skills and motor behaviour. These will be designated "unlearned" behaviours (Curry 1987) and will be reviewed in the second section of this chapter. Firstly, however, the major findings concerning "learned" behaviour will be considered.

2:2 SECTION A: LEARNED BEHAVIOUR

The early report by Hebb (1947) that rats reared as pets learned mazes more rapidly than rats reared in laboratory cages and his subsequent assertion that it was "the richer experience of the pet group during development (that) made them better able to profit by new experiences at maturity" (Hebb 1949 p 298) provided the impetus for a major effort to investigate and understand the effects of differential experience on subsequent learning, or problem-solving behaviour.

Since this seminal work a wide range of tests linked with several types of learning paradigms have been employed in the literature and will be described in some detail in this section. For ease of organisation the material will be further subdivided into the following:

1. MAZE LEARNING
    - Hebb-Williams
    - Lashley III Type Maze
    - Other
2. DISCRIMINATION LEARNING
    - Brightness
    - Pattern
    - Spatial
    - Tactile

3. DISCRIMINATION REVERSAL LEARNING

4. AVOIDANCE LEARNING

   - Active
   - Passive

5. SKINNER BOX CONDITIONING

   - Simple Procedures
   - Complex Procedures

2:2.1 MAZE LEARNING

a) Hebb-Williams Maze

In 1946, Hebb and Williams described a method of rating animal intelligence using a closed field test apparatus. This, it was claimed, provided the first method of analysing the *quality* of performance coupled with sytematically comparable scores for different subjects. This first "Hebb-Williams" maze was refined and standardised by Rabinovitch and Rosvold in 1951 and in this later form bases its quantitative score on qualitative analyses of performance on twelve different tasks. Within the EC/IC literature, it is the latter version of the maze and its concomitant procedures which are typically used by researchers, albeit in an idiosyncratically modified form.

Since the early investigations of Hebb (1947,1949) a large number of papers describing the effects of differential environments on Hebb-Williams maze performance has appeared in the literature. Table 2:1 lists chronologically 40 studies that have found superior maze performance in animals reared in complex environments, when compared with animals raised in isolation, or in socially housed conditions[1]. Initially, this EC performance superiority was interpreted as a difference in "intelligence" between the groups (Hebb 1947; 1949; Hebb and Thompson 1954). However,

[1] There is one further study (Yamamoto et al 1988) which could also be included in Table 2:1. Unfortunately this paper is written in Japanese so few details were available, other than the fact that raising animals in isolation produced animals whose performance in the Hebb-Williams maze resulted in more errors than animals raised in socially enriched groups. Interestingly, extra-cage stimulation did not enhance group housing and indeed, produced animals whose performance in the Hebb-Williams was less efficient than their socially enriched counterparts.

REFERENCE	AGE	STRAIN	GENDER	COMPARISON	TRAINING AND TESTING PROCEDURES
Hymovitch 1952	27-79	Hooded	M	EC-IC	6 Training probl. 24 Subtests
Forgays and Forgays 1952	26-90	McGill	M	EC-SC	7 days training 24 test probl.
Smith 1956	18-90	Long Evans	M	EC-SC	prelim R/R training 24 tests probl.
Eingold 1956	55-75	no details	M F	EC-SC	no details no details
Cooper and Zubek 1958	25-65	McGill Bright McGill Dull	M F	EC-SC control	prelim R/R training 12 test probl.
Dawson and Hoffman 1958	0-30	Wistar	M F	EC-SC	3 days training 4 days testing
Woods 1959	23-54	Sprague Dawley	M F	EC-IC	9 days training 3 days testing
Woods et al. 1960	21-90	Sprague Dawley	M F	EC-SC	prelim R/R training 12 test probl.
Woods et al. 1961	25-155	Sprague Dawley	M F	EC-IC	6 days training 12 test probl.
Forgays and Read 1962	0-109 (various)	Rutgers Albino	M	EC-IC	7 days training 12 days testing
Denenberg and Morton 1962	0-50	Wistar	M F	EC-IC	prelim R/R training 12 test probl.
Schwartz 1964	0-96	Hooded	M F	EC-SC	prelim R/R training 12 test probl.
Hughes 1965	33-66	Holtzmann	M	EC-IC	prelim R/R training 7 test probl.
Schweikert and Collins 1966	25-75	Wistar		EC-SC-MC	9 days training 12 dyas testing
Ravizza and Herschberger 1966	0-19	Charles River	M F	EC-SC (Handled)	Adapted R/R 18 test probl.
Nyman 1967	30-80	Hooded	M	EC-SC	10 days training 2 test probl.
Brown 1968	20-100 (various)	Long Evans	M	EC-IC and others	21 days training 12 test probl.
Denenberg et al. 1968	0-50	Purdue Wistar	F	EC-SC	3 days training 12 test probl.
Lavallee 1969	21-90	Albino and Hooded	M F	EC-IC	prelim R/R training tested in light and tested in dark

Table 2:1 Chronological listing of all studies which have found superior performance in enriched animals in the Hebb-Williams maze.
Key for this, and all other tables in this chapter:
SEC=Superenriched Condition
EC= Enriched Condition
IC= Impoverished Condition
SC= Standard Condition
M= Male F= Female
AGE= Treatment Age
< and > indicates direction of results
R/R= Rabinovitch and Rosvold 1951

REFERENCE	AGE	STRAIN	GENDER	COMPARISON	TRAINING AND TESTING PROCEDURES
Sturgeon and Reid 1971	21-81	Hooded	M	EC-IC and SEC	modified R/R training 36 test probl.
Tanabe 1972	25-60	Wistar	M	EC-IC	no details no details
Wells 1971	21-45	no details	no details	EC-IC	no details no details
Wells et al. 1972	21-45	Holtzmann	M	EC-IC	6 practice probl. 12 test probl.
Smith 1972	0-55 21-55	Carworth Europe	M F	EC-SC EC-IC	long training period adapted from R/R 12 test probl.
Cummins et al. 1973	21-509	Wistar	M	EC-IC	no details no details
Will et al 1976	0-65 21-40	Berkeley S1	M M F	EC-SC EC-IC	prelim R/R training 12 test probl.
Ivinskis and Ivinskis 1976	22-46	Albino	M F	EC-control	prelim R/R training 2 test probl.
Will et al. 1977	36-	Fisher Berkeley S1	M	EC-IC	11 days training 12 test probl.
Kelche and Will 1978	125-165	August	M	EC-IC	prelim R/R training 12 test probl.
Rosenzweig and Bennett 1978	various	Berkeley	M	EC-SC	no details no details
Celedon et al. 1979	21-98	Sprague Dawley	M F	EC-IC	prelim R/R training 12 test probl.
Ivinskis and Homewood 1980	various	nodetails	M F	EC-SC	prelim R/R training 2 test probl.
Renner et al. 1981	21-90	S1	M	EC-IC	no details no details
Chadha and Rose 1981	21-51	Hooded	M F	EC-IC	8 days training 1 test probl.
Kiyono et al. 1981b	21-51	Sprague Dawley	M	EC-SC	prelim R/R training 12 test probl.
Shibagaki et al. 1981	21-51	Sprague Dawley	M	EC-IC-SC	prelim R/R training 12 test probl.
Seo et al. 1982	23-53	Sprague Dawley	M	EC-IC-SC	prelim R/R training 12 test probl.
Dell and Rose 1986	21-49	Hooded	M	EC-IC	prelim R/R training 6 test probl.
Pappas et al 1987	25-60	Wistar	M	EC-IC	prelim R/R training 12 test probl.
Venable et al 1988	10-24	Gray (AXC)	M	EC-SC	prelim R/R training 12 test probl.

Table 2:1 continued.
For key, please see page 64.

under certain circumstances, IC performance has been found to be equivalent (Eingold 1956; Woods et al 1961; Hughes 1965; Reid et al 1968; Aubrecht 1974) or indeed superior to EC performance (Hymovitch 1952; Forgays and Forgays 1952). Table 2:2 comprises those few studies where no EC performance superiority has been found. These studies are particularily interesting, as they suggest that the use of the term "intelligence" (Hebb 1947) may be misleading and offer other explanations for the performance of differentially housed animals in the Hebb-Willams maze.

Hymovitch (1952) was the first researcher to replicate Hebb's (1947) findings of improved maze performance in animals with greater infant experience, using larger numbers of animals and more carefully controlled conditions. Animals were raised in either a "free" environment (FE) which provided extensive opportunities for experience, (it contained a variety of alleys, inclined runways, small enclosed areas and apertures), small mesh cages, enclosed activity wheels or stovepipe cages (SP). The last condition restricted both social experience and visual perception. Testing in the Hebb-Williams maze revealed that the FE group was clearly superior to the SP group over 24 test problems. However, when the Hebb-Williams test apparatus was rotated 90^o clockwise, FE animals made significantly more errors than the SP group. Hymovitch interpreted this as evidence that the problem-solving behaviour of the FE rat was more dependant on a "wider sensory environment" than that of the SP rat. This finding, that under certain conditions, IC animals perform better than their FE counterparts, was replicated by Forgays and Forgays (1952). They initially found superior Hebb-Williams performance in FE animals, when compared with animals raised in isolation, but when the apparatus was rotated, FE animals made more errors than the IC animals. This was explained by the greater use of visual distance cues in the FE rat, as a result of early experience. Indeed, when extra-maze cues were removed by the simple expedient of suspending a black cloth one foot beyond the perimeter of the Hebb-Williams maze in a later experiment (Ravizza and Herschberger 1966), forcing subjects to rely heavily on motor cues, animals that had experienced motor restriction in their early experience exhibited inferior performance when compared with non-restricted subjects.

REFERENCE	AGE	STRAIN	GENDER	COMPARISON	TRAINING AND TESTING PROCEDURES
Hymovitch 1952	27-79	Hooded	M	EC-IC	trained/tested 24 probl. rotated probl.
Forgays and Forgays 1952	26-90	McGill	M	EC-SC	7 days training rotated test problem
Eingold 1956	35-55 75-95	no details	M F	EC-SC	no details no details
Woods et al. 1961	25-60	Sprague Dawley	M F	EC-SC	Prelim R/R training high and low drive groups
Hughes 1965	33-66	Holtzmann	M	EC-IC	Prelim R/R training 7test probl.
Reid et al. 1968	21-81	Hooded	M	EC-IC	high degree training and deprivation 24 test probl.
Aubrecht 1974	21-66	Holtzmann	no details	IC-SC	no details no details

Table 2:2 Chronological listing of all studies where no EC performance superiority has been found.
For Key please see page 64.

The use of extramaze cues, as a problem-solving strategy, is not the only explanation for the typical finding of FE superiority in the Hebb-Williams apparatus. Zimbardo and Montgomery (1957) speculated that the problem-solving ability shown by the FE animals might be due to the fact that the test situation had relatively less novelty for them. As a result, FE animals, it was suggested, engaged in less exploratory activity and hence made fewer errors. Indeed, Woods (1959) reported that some animals would solve the maze problem, but would then wander back through the maze before returning to eat the food and thus terminate the trial [2]. In these cases, total error score was not just a reflection of the difficulty the animal had in solving the maze, but rather a combination of this, and exploratory behaviour. In a later experiment (Woods et al 1960), both exploration and error scores were examined. High correlations were found between these measures, with restricted females exhibiting high levels of exploration suggesting that exploratory differences rather than "intelligence" differences were a major factor in the characteristic finding of FE animals' problem-solving superiority. Furthermore, adaption and preliminary training *increased* the amount of exploratory behaviour in the restricted groups, and *decreased* it in the 'free' groups, thus maximising the differences between the groups.

In a further experiment, (Woods et al 1961) exploratory behaviour was experimentally mediated, in the restricted group, by establishing a strong and competing drive. Under these conditions, restricted animals' performance was equivalent to that of the FE group, suggesting that exploration was a realistic explanation for the perceived IC deficit in Hebb-Williams performance. Indeed, this hypothesis was futher substantiated by Reid et al (1968) who found isolated subjects to be as efficient as enriched animals in solving maze problems, when both the amount of pretraining, and degree of deprivation were extraordinarily high. Furthermore, Reid et al (1968) suggested that the main difference between the groups' early experience was the *relevance* of that experience. For example, the early experience and learning of the enriched group may well transfer positively to certain kinds of problems, whereas the early experience and learning of the

[2] More recently, Holson (1986) has argued that IC animals turn away prior to reaching the goal box, despite knowing where it is, that is they actively avoid the goal box and its food reinforcement. This he attributes to a form of neophobia, that these animals are wary of eating in a novel environment.

isolated group may not tranfer to those same problems. They speculated that the transfer of learning to habituate to irrelevancy was the factor that most easily accounted for the differences in problem solving aptitude of the two groups, a hypothesis that was later examined by Dell and Rose (1986). These authors reported that the acquisition slope for Hebb-Williams maze performance did not differentiate EC from IC animals, but that EC-IC differences were due to the impaired asymptotic performance of the IC subjects. This, it was felt, was due to the inadequacy of response inhibition in the IC animals, such that having established a route through the maze (initial learning), irrelevant diversions were maintained, despite their redundancy. Interestingly, in this paper, no differences in exploratory behaviour, as measured by number of rears in the maze, were found between the groups.

As well as examining the underlying causes of the differential maze performances of the enriched and impoverished animals, other factors such as quality of experience, age of onset of experience, and duration of experience have also been investigated in the literature.

With respect to the quality of experience, typically the "enriched", "complex" or "free" environment consists of ten or more animals living in a large cage in which there is a variety of stimulus objects (Rosenzweig and Bennett 1977). Often there is also a variety of visual and auditory stimulation around the cage; thus this type of environment includes social stimulation, stimulation from inanimate objects with which the animals can have direct contact and stimulation through distance receptors by objects with which the animals have no direct contact. Each of these dimensions has been investigated and the effects on Hebb-Williams performance noted. Taking social stimulation first, it is apparent that *degree* of socialisation, as measured by numbers of animals, is an important factor in the enrichment effect. Aubrecht (1974), one of the few researchers to find no differences between his groups, investigated the importance of "the social factor" (sic) by raising animals either in isolation, or in pairs. His lack of significant differences is not surprising, however, considering that Brown (1968) housed his "restricted" group in threes. In this latter experiment, socially housed animals raised in groups of 25 in a bare enclosure, performed signif-

icantly better than the restricted animals. Maximal effects, however, require both social contact and object interaction. Brown (1968) found animals housed in a complex environment, in groups of 25, performed significantly better in the Hebb-Williams maze, when compared with 25 socially housed animals. Rosenzweig and Bennett (1978) have also found superior EC performance, when compared with socially housed controls, as have Forgays and Forgays (1952). The only paper to find no differences between socially housed animals and socially housed animals with object interaction was Aubrecht (1974). However, his group sizes were small, with animals being housed in pairs, which may well have biased the results. Interestingly, frequently changing the objects in the enriched environment to provide additional enrichment (Sturgeon and Reid 1971) does not significantly alter problem-solving performances in these differentially enriched groups (Ivinskis and Ivinskis 1976).

The importance of extra-cage stimulation has been investigated by Lavallee (1969,1970). Rats given daily slide shows from weaning to 80 days of age were superior problem solvers when compared with control rats exposed to blank screens. However, Rosenzweig and Bennett (1976) report an unpublished study by Ricard, which failed to replicate these findings and indeed state "any investigator who believes the extracage stimuli to be effective in determination of EC-IC differences is encouraged to present evidence that this is more than fantasy" (Rosenzweig and Bennett 1976 p 194). In addition superior performance in enriched animals has been found in animals blinded at infancy, or indeed at maturity, for which extracage visual experience would have been irrelevant (Hebb 1947; Hymovitch 1952) which lessens the importance of this particular dimension of experience on the general finding of superior EC performance in the Hebb-Williams maze. Finally quality of experience has also been investigated by Woods (1959), who found that animals initially placed in isolation at weaning and transferred to an enriched environment at 66 days of age, performed significantly better than animals that had remained in the isolation cages.

Briefly then, in general, the greater the social and perceptual experience, the better the per-

formance of the animals concerned in the Hebb-Williams Maze. Furthermore, the behavioural effects of isolation can be reversed, and Hebb-Williams performance improved (Woods 1959).

A second factor, which might influence an animal's maze performance, namely age at which the experience was initiated, has also been examined in the literature. Hymovitch (1952) was the first to report that animals exposed to free environmental experience early in life (30-75 days) were superior to those that had had this experience when mature (85-130 days). Subsequent experiments have confirmed this finding, although there has been some debate as to exactly which period is the most effective, within this "early" time scale. Eingold (1956) for example, found that groups receiving free environmental experience at a mean age of 55 days, performed significantly better than those receiving it at 35 or 75 days. Forgays and Read (1962) on the other hand, found no differences between groups exposed to differential experience between either 22-43 days or 44-65 days. Preweaning enrichment has also been found to reduce error scores (Dawson and Hoffman 1958; Forgays and Read 1962; Denenberg, Woodcock and Rosenberg 1968; Will et al 1976; Ivinskis and Homewood 1980; Venable et al 1980), although according to Forgays and Read (1962) this is less effective than experience during adolescence. More recently (Rosenzweig and Bennett 1978) in six experiments in which differential experience started at 85, 101 or 123 days of age, significant effects have been found between animals exposed to EC versus SC experience. Finally, when preweaning (Days 10-24) enrichment is combined with training, animals' performance is superior to that of littermates exposed to postweaning enrichment (Venable et al 1988), suggesting that the quality of experience must also be taken into account when the effects of age at which experience occurs are investigated. Overall therefore, although it appears preferable to expose animals to differential experience during adolescence, enrichment will produce effects in preweaning, adolescent or mature animals.

Duration of exposure has also been investigated with respect to Hebb-Williams performance. Eingold (1956) exposed animals to differential experience at different ages, for either 10 or 20 days, and reported that length of exposure was not a significant variable. Nyman (1967) on the

other hand, exposed animals to either 8 hours or 1 hour of experience a day, for either 30-40, 50-60 or 70-80 days, and found that "more experience" was more effective than "less experience" over all three age periods. It appears from these papers that a minimum of experience is required to produce the EC-IC effects, but that this minimum is surprisingly small.

In summary then, the majority of the experiments in this field has found that enriched animals perform better in the Hebb-Williams maze than either socially housed or isolated animals. Enrichment produces an animal that is better able to use extramaze cues, (Hymovitch 1952; Forgays and Forgays 1952) whereas restriction induces higher levels of exploration (Woods 1959; Woods et al 1960; 1961) and inadequate response inhibition (Reid et al 1968; Dell and Rose 1986). Indeed, it appears that, as Greenough (1976) says, "differentially reared animals have learned different techniques for dealing with their environment".

b) Lashley III Maze

The Lashley III Maze (Lashley 1929) typically comprises a rectangular box, subdivided lengthways by three partitions, thus forming four alleyways with eight culs de sac. Openings in the partitions and from alternate sides of the box where the start and goal boxes are located, allow the animals to trace a path through the maze. Errors are scored whenever the animal enters the blind alleys, demarkated on the floor of the maze by lines.

Of the 16 studies examining the effects of differential environments on performance in this type of maze, only three have found no significant differences between the groups. Table 2:3 lists chronologically those studies that have found EC animals to be superior in the Lashley III maze, whereas Table 2:4 details those few studies where performance of the groups was equivalent. Interestingly, no studies have reported evidence of IC superiority in this apparatus.

As with the Hebb-Williams literature, it is those atypical studies, which have found no significant differences between the groups, which require some explanation. Indeed, both Peeke et al (1971)

REFERENCE	AGE	STRAIN	GENDER	COMPARISON	TRAINING AND TESTING PROCEDURES
Ray and Hochhauser 1969	21-85	Zivic Miller	M F	EC-IC(p)	4 days 14 days
Greenough et al. 1970	25-81	DBA2J Mice	M	EC-IC	5 days 3 days
Bennett et al. 1970	25-55 60-90	S1 S1	M M	EC-IC EC-IC	no detail no detail
Rosenzweig 1971	30 days	S1	M	2hrsEC-IC	no detail no detail
Riege 1971	310-360	S1	M	EC-IC	5 days 4 days
West and Greenough 1972	21-51	Long-Evans	M	EC-IC	5 days 5 days
Freeman and Ray 1972	28-88	Zivic-Miller F344/FMai	M F	EC-IC(p)	4 days 14 days
Greenough et al. 1972a	22-52	Long-Evans	M	EC-IC	8 days 5 days
Greenough et al 1972b	25-55	Mice	M	EC-IC	no detail spaced and massed trials
Greenough et al. 1973	22-52	Long-Evans	M	EC-IC	5 days 2 days
Bernstein 1973	21-84	Wistar	M	EC-IC	no detail trials to criterion
Warren 1985	no details	Mice	no details	EC-IC	no details
Pappas et al 1987	25-60	Wistar	M	EC-IC	no details 10 days

Table 2:3 Chronological listing of all studies with an EC superiority in the Lashley III Maze.
For key, please see page 64. (NB IC(p)=IC animals housed in pairs)

REFERENCE	AGE	STRAIN	GENDER	COMPARISON	TRAINING AND TESTING PROCEDURES
Le Beouf and Peeke 1969	21-52	K	M F	EC-IC(p)	no detail trials to criterion
Peeke et al. 1971	51-70	Long-Evans	M	EC-IC	3 days trials to criterion
Warren et al. 1982	600-750	C57BL/6J Mice	M	EC-IC	no detail 10 days

Table 2:4 Chronological listing of all studies with no EC superiority in the Lashley III Maze

and Le Beouf and Peeke (1969) suggest that their results can be accounted for in terms of "the time required to produce modifications in adult learning ability" by which they mean a critical length of time before the effects of enrichment manifest themselves in the performance of enriched animals. In their studies, this is operationalised in terms of the onset and duration of differential experience. Considering the studies in Table 2:3, however, this explanation loses credence, as several researchers (Bennett et al 1970; West and Greenough 1972; Greenough et al 1972a; 1972b; 1973) have also used similar "time" periods to those of Peeke et al (1971) and Le Beouf and Peeke (1969), and found significant differences between the groups. Age of onset of differential experience may account for the findings of the third study that has reported equivalent performance in EC-IC animals (Warren et al 1982), however, in this study mice [3] were exposed to environmental experience between 600-750 days. In this instance there are no parallels in Table 2:3 although Riege (1971) has found significant differences between animals exposed to EC-IC between 310-360 days, a time when the rat is considered to be "old".

Typically in the literature, age of exposure to differential environments has not been found to influence Lashley III maze performance in those studies finding significant EC-IC differences. Probably the first report of manipulating two age periods, was that of Bennett et al (1970). They reported significant differences between EC-IC groups' initial errors, at both 25-55, and 60-90 days of exposure. Futhermore, Rosenzweig (1971) suggests that maze performance is enhanced by experience in exploring open fields, which may be gained at any age, although it should be noted that this improvement in performance might not be equally effective at different ages.

Strain differences, and their effects on Lashley III maze performance have also been examined with no differences being found betweeen Zivic Miller and F344/fMai animals (Freeman and Ray 1972). Indeed, as can be seen from Table 2:3, a variety of strains has been used and significant differences found between EC-IC animals. It is therefore unlikely that strain differences have caused the lack of significant findings in the three studies described in Table 2:4.

[3] It is worth noting here that this result is not just a reflection of species differences, as enrichment effects have been found in a variety of different species (Renner and Rosenzweig 1987).

The nature of environmental enrichment has also been examined in the Lashley III maze literature. Enrichment consists of both social and perceptual experience and the nature of social experience alone has provided a possible explanation for understanding enhanced EC performance in the Lashley apparatus. An early view was that social enrichment with large groups of animals produces animals that do not differ from socially and perceptually enriched animals (Bernstein 1973). Reducing the numbers to two or three animals typically removes any enrichment effects (Ray and Hochhauser 1969; Rosenzweig 1971; Freeman and Ray 1972), which has led Rosenzweig (1971) to suggest that the Lashley III maze is sensitive to enrichment of experience above the colony level (p320). However, under certain testing conditions, where spaced practice trials are employed, a degree of social enrichment (housing in pairs) produces performances equivalent to animals housed in socially and perceptually enriched environments (Greenough et al 1972b). This suggests that degree of enrichment produces animals with different capacities to process or store information and that if the behavioural test is appropriate, minimal amounts of social enrichment will produce superior performance in the maze, when compared with animals raised in isolation. Indeed, the interaction between behavioural testing and the nature of enrichment may well explain those studies where no EC-IC effects have been found.

Other than differential processing and storing capacity, a variety of explanations has also been put forward to explain the EC-IC performance differences in the Lashley III maze. Firstly, the notion that familiarity with environments similar to mazes may improve EC performance (Riege 1971) is probably correct, but, as Greenough (1976) points out, not very useful for interpretation of changes in EC behavioural capacity [4]. Secondly, the idea that fear or reactivity to either the experimenter (McCall, Lester and Dolan 1969) or to the test situation (Myers and Fox 1963) in the IC animal may produce behavioural deficits which account for the EC-IC performance differences, has received limited support. As Greenough et al (1972a) point out, pretraining should make the groups homogenous with respect to these factors, prior to the testing phase

[4] Presumably as effects are observed in a variety of different learning procedures that are dissimilar to enriched environments.

[5]. In situations with little or no pretraining, however, IC reactivity may well contribute to inferior maze performance. Thirdly, Freeman and Ray (1972) state that, following the lack of observed differences in open field activity in their study, differences in performance in the Lashley III maze cannot be accounted for in terms of general activity differences. However, they do acknowledge that increased duration of exposure to isolation results in increased emotionality, as measured by defecations in the Open Field, which would result in "freezing" and this in turn would interfere with solving the Lashley III maze [6]. Fourthly, West and Greenough (1972) suggest that their results represent either an enhancement of some normal developmental process in the enriched animals, or a retardation of such a process in the isolated rat. However, in a further experiment, as outlined above, (Greenough et al 1972b) it was suggested that the consistently poorer performance of the isolated animal might reflect deficits in information storage. This fifth hypothesis has since received further support (Greenough et al 1973), although, whether the EC-IC differences in memory storage parameters were related to differences in attentional mechanisms involved in memory, or to differences in memory storage *per se* was not specified. Finally, in a recent paper (Pappas et al 1987) in which newborn male rats were depleted of forebrain norepinephrine (NE) by systemic 6-hydroxydopamine injection, isolated rearing was found to impair Lashley maze performance in control animals but not in the injected rats. This suggests that intact NE terminals are important in the mediation of isolation-induced deficits and offers a biochemical level of analysis, reflecting a growing trend in the EC-IC literature to investigate the causes of behaviour differences at a neurochemical level.

To summarise, in general, environmental enrichment produces an animal with enhanced Lashley III maze performance. To date, in the literature, no firm explanations of what causes this superior performance have been proffered, although both improved information storage and familiarity with problem-solving environments in the enriched animal and increased reactance, emotionality and deficits in information storage or NE functioning in the IC animal have all been suggested

[5] Although it is not clear whether it does always eliminate the differences.

[6] It should be pointed out, however, that other researchers have found differences in activity in the open field, research which is reviewed in section 2:3:1 of this review.

as important in mediating the behavioural differences between the groups.

c) Other Mazes

This section will review the behavioural testing of differentially reared animals, in mazes other than the Hebb-Williams and Lashley III paradigms. Within the literature, there has been a variety of different types of maze employed, including Y mazes (Zimbardo and Montgomery 1957; Montgomery and Zimbardo 1957; Ehrlich 1959; Forgays and Read 1962; Einon and Morgan 1978b), T mazes (Hymovitch 1952; Forgus 1955a; 1955b; Goldman et al 1987), L mazes (Watson and Livesey 1982), inclined or elevated mazes (Bingham and Griffiths 1952; Forgus 1954; Luchins and Forgus 1955), multiple U mazes (Myers and Fox 1963), radial mazes (Einon 1980b; Juraska et al 1984; Van Gool et al 1985; Pacteau et al 1989), Dashiell mazes (Ehrlich 1959; Bennett et al 1970), symmetrical mazes (Gonzales and Davenport 1972; 1973; Davenport 1974-1975; Joseph 1979; Joseph and Gallagher 1980), Warner-Warden mazes (Bingham and Griffiths 1952) complex mazes (Holson 1986) and water mazes (Duke and Seaman 1964; Whishaw et al 1986; Saari et al 1990a; 1990b). Results from these paradigms are *less consistent* than those detailed in the previous two sections, although still producing highly significant results. Of these studies, four (Pacteau et al 1989; Whishaw et al 1986; Saari et al 1990a; 1990b) have employed enrichment as a therapeutic environment following noradrenaline depletion or hippocampal lesions and thus effects on intact animals may be confounded by the control procedures employed (sham operations or placebo injections). As a consequence, these studies will not be included in this review.

Table 2:5 lists 17 studies that have found animals with enriched experience to be superior performers, whereas Table 2:6 lists 9 studies where no differences have been found between the groups. As with the Lashley III literature, and worth highlighting, there are no studies reporting IC superiority. This inconsistency is perhaps unsurprising, considering the variety of paradigms employed. Inspection of Tables 2:5 and 2:6 reveals a large variation between studies in terms of strain of subjects, age at onset of treatment, nature of differential experience employed and

REFERENCE	AGE	STRAIN	GENDER	COMPARISON	MAZE TYPE	TRAINING AND TESTING PROCEDURES
Bingham and Griffiths 1952	21-51	Albino	M F	FE-SP	inclined Warner-Warden	4 days 8 days T to C
Forgus 1954	24-84	Hooded	M	CVP-CV-M	elevated Einstellung	no details 2 pathways free choice
Forgus 1955a	25-85	Hooded	M	VM-V	11 unit T	initial learning no visual cues
Forgus 1955b	25-85	Hooded	M	VM-V	11 unit T	light on dark
Luchins and Forgus 1955	18-70	Hooded	F	EC-SC	elevated	40 trials harnessed direct path
Zimbardo and Montgomery 1957	21-46 21-71 21-121	Wistar	M F	FE-SC	Y	no details
Myers and Fox 1963	21-261	Colgate		SC-IC	multiple U	no details T to C
Duke and Seaman 1964	21-121	Albino	M F	FE-SC	water/6 choice	no details 7 days
Bennett et al. 1970	21-51	S1		EC-IC	Dashiell	no details no detaisl
Joseph 1979	21-100	Holtzman	M F	EC-IC	symmetrical	no details 7 maze probl
Joseph and Gallagher 1980	19-72	Zivic Miller	M F	EC-IC	symmetrical	20 trials 1 probl.
Einon 1980	21-45 23-45 23-60	Wistar Hooded Lister	M F F F	EC-IC SC-IC SC-IC	radial radial radial	8 days 9 days
Watson and Livesey 1982	23-33 23-53 23-74	Albino	M	EC-IC	L	none 4 trials a day
Juraska et al. 1984	23-53	Long Evans	M F	EC-IC	17 arm radial	4 days 24 days
Warren 1985	no details	Mice	no details	EC-IC	Stone	no details
Holson 1986	21-111	Long Evans	M	EC-IC	Complex	None 3 trials
Goldman et al 1987	6 months 12-14 months 24-26 months	Sprague Dawley	M	EC-SC (14 unit T)	Stone 25 trials	Runway training

Table 2:5 Chronological listing of all studies with an EC superiority in mazes other than the Hebb-Williams and Lashley III mazes. (FE=Free Environment;SP=Stove Pipe;CVP=Complex Visual and Proprioceptive; CV=complex Visual; M=Minimal Visual; VM=Visual Motor; V=Visual;T to C= Trials to Criterion)

REFERENCE	AGE	STRAIN	GENDER	COMPARISON	MAZE TYPE	TRAINING AND TESTING PROCEDURES
Hymovitch 1952	27-79	Hooded	M	FE-SP	T	2 days 3 days
Montgomery and Zimbardo 1957	25-50 25-75 25-125	Wistar	M F	NC-BD-SBD	Y	no details 4 trials
Ehrlich 1959	21-73	Hooded	M	EC-SC	Y Dashiell	no details 3 days
Forgays and Read 1962	0-109 various	Albino	M	FE-SC	Y	2 days
Gonzales and Davenport 1972	30-67	Holtzman		EC-IC	symmetrical	no details 4 probl.
Gonzales and Davenport 1973	30-67	Holtzman		EC-IC	symmetrical	no details 3 probl.
Davenport 1974-1975	36-70	Holtzman		SEC-IC	symmetrical	4 practice 4 probl.
Einon and Morgan 1978	23-45	Hooded Lister	F	SC-IC	Y	none used 11 trials
Van Gool et al. 1985	140-189 784-833	Brown Norway	M	EC-SC	radial 8 arm	5 days 20 days

Table 2:6 Chronological listing of all studies with no EC superiority in mazes other than the Hebb-Williams and Lashley III mazes. (NC=Normal Cage;BD=Behaviourally Deprived;SBD=Sensorily and Behaviourally Deprived; SEC=Super Enriched condition)

procedures and techniques used in data collection. This suggests that there is little likelihood of explaining why EC superiority has been found in 17 studies but not in nine other studies, in terms of *one factor*. In fact, considering those studies where no differences have been found between the groups, several reasons emerge which could explain the nonsignificant results of individual papers.

The first report of equivalent performance between differentially housed groups was that of Hymovitch (1952). In this experiment animals were tested for rote learning ability in an enclosed T maze paradigm, after having had extensive Hebb-Williams training. This procedural detail is in fact important, as Rosenzweig and Bennett (1977) have suggested that "the differences in problem solving ability brought about by differential experience can be overcome during the course of prolonged testing" (p172), an idea that has subsequently been invoked by Rose, Dell, Love and Davey (1988). Indeed, behavioural differences were found between the groups in the earlier Hebb-Williams problem solving task, in Hymovitch's study.

The second study to report no differences between groups housed in normal mesh "behavioural deprivation" cages or "sensory and behavioural" deprivation cages, was that of Montgomery and Zimbardo (1957). In this experiment, a standard Y maze was employed, with ***exploration*** being measured, by counting the number of maze sections traversed per minute. It was predicted that, as exploration is dependant upon exteroceptive stimulation (Zimbardo and Montgomery 1957), differences should only appear if there were a differential increase in novelty of the testing situation. In this instance the Y maze offered *all* groups the opportunity to explore an environment which was richer than their cage environment, thus obviating any exploration differences. In fact in a second study (Zimbardo and Montgomery 1957), animals raised in normal cages were found to explore more in a Y maze, than animals from a free environment, corroborating this theory [7]. Interestingly, Luchins and Forgus (1955) reported the opposite effects, in that their experimental animals, reared in a large and stimulating environment, were more active than their

[7] However, it should be pointed out that it might be degree of difference from natural environment that is the important factor, an hypothesis not explored by these authors.

control animals. However, it appears that these latter results can be attributed to a "handling" factor, as Luchins and Forgus (1955) handled their experimental animals, which has been found to significantly increase exploration (Ehrlich 1959).

A third study that reported no significant differences between differentially housed groups used a similar paradigm, namely exploration as measured by number of units entered, but this time in two test paradigms, the Y maze and the Dashiell maze (Ehrlich 1959). In this instance lack of significant differences were explained by small group sizes and large variability in the data, which rather lacks credence as an explanation. The tendency was, however, for the restricted rats to explore more than the free environment rats, a tendency that was consistent across both maze paradigms. In a fourth study, which also measured activity in a Y maze, Forgays and Read (1962) reported no differences between the groups. In this instance there are no obvious methodological reasons for the lack of significant findings. Finally, the Y maze has also been employed as an alternation test (Einon and Morgan 1978b) with total number of alternations (calculated seperately for each rat) revealing no significant differences between those animals housed in social groups of five and those reared in isolation prior to testing. In a locomotor activity task, however, IC animals were more active than their social controls. These authors have therefore argued that caution should be exercised "in relating hyperactivity and reduced alternation to a unitary deficit in an inhibitory system" (p151). That is, whatever is causing differences in EC/IC performance, advocating one underlying mechanism such as inappropriate levels of inhibition is not an adequate explanation for what is emerging as a complex behavioural and neurochemical difference (Pappas et al 1987) between these differentially housed animals.

Other than the early papers investigating the Y maze, a group of studies in the 1970's (Gonzales and Davenport 1972; 1973; Davenport 1974-1975) using a symmetrical maze, has also reported a lack of differences between enriched and impoverished rats. These negative results are unusual, considering the similarity of the symmetrical maze to the Hebb-Williams maze, the latter maze typically producing superior EC performance (see section 2:2.1a). One explanation for their

findings lies in their procedure. As Davenport (1976) points out their food deprivation schedules were moderately severe and their rats well adapted prior to the task. Both high motivation such as is induced by food deprivation and extensive pre-training have been found to obviate EC-IC differences in Hebb-Williams performances (Woods et al 1961; Reid et al 1968). Consequently these factors might also have influenced Davenport et al's results. Indeed, both Joseph (1979) and Joseph and Gallagher (1980) have since found significant differences in favour of enriched animals using the symmetrical maze, suggesting that differences in procedures may well greatly modify results.

The final paper in Table 2:6, which has reported no significant differences between the groups, is that of Van Gool et al (1985). In this study differential experience was given to animals unusually late in life (either 140-189 days or 784-833 days approx.) Age of onset may well be an important factor, as Warren et al (1982) using geriatric mice, also failed to find significant differences between EC-IC animals in the Lashley III maze. Furthermore, in this experiment, small groups of animals (N=8 and N=5) were housed together in the enriched condition. This in itself may well have contributed to the lack of significant differences, as Aubrecht (1974) found no differences between *small* groups of animals housed with or without objects, in a Hebb-Williams paradigm, and Van Gool et al's control groups comprised similarly small sample sizes (N=4).

In summary then, most of the studies reporting equivalent performances between the groups have methodological, or procedural anomalies which might explain their findings. However, care must be taken in interpreting the data in all the studies in this area, because of the wide procedural differences employed, as well as the diversity of dependant variables studied.

With respect to those studies in Table 2:5 where significant differences have been found between the groups, several hypotheses have been postulated to explain the results. These will be outlined briefly, below.

Hebb (1949) suggested that it was the richer experience of rats raised as pets during their early development which made them better able to profit from new experiences at maturity,

compared with laboratory housed animals. Furthermore, Bingham and Griffiths (1952), the first researchers to find significant differences between their groups using an inclined maze, agreed that their findings supported Hebb's hypothesis. Extending this hypothesis, Forgus (1954) pointed out that early experience and learning were important determinants of cognitive ability in the adult rat, and that the quality of the infantile experience determined the "kinds and numbers of hypotheses" that the animals could test when problem solving. In later papers (Forgus 1955a; 1955b) the relative importance of the early experience was found to depend on the nature of the problem requirements. In particular, it was postulated that the relationship between the kind of early experience and the demands of the problem task was the most important factor in mediating the typical EC results. Indeed, Luchins and Forgus (1955), when considering Hebb's hypothesis that animals reared under different environmental conditions develop nervous systems which are organised differently, suggest that their results support the contention that differential past experiences result in the development of different kinds of "experienced beings".

An alternative hypothesis, however, is that of Zimbardo and Montgomery (1957). For these authors past experience affects the "complexity" of subjects and therefore the range of stimuli to which they will respond. Superior EC maze performance, in their opinion, is due to the low exploration of the enriched animal, caused by the relatively decreased novelty of the test situation, when compared with its IC counterpart. In other words, IC animals have a high exploratory drive, which is increasing their error scores in the maze, so that performance differences reflect an IC deficit, rather than an EC enhancement. Indeed, throughout the literature, the isolate maze-deficit has been explained in terms of the hyperactivity often seen in such animals (Einon and Morgan 1977; Morgan 1973; Smith 1972) a syndrome which according to Holson (1986) "could result in cue inattention and consequent poor performance" (p191). A second and related hypothesis, which again suggests that superior EC performance is due to an IC deficit, rather than EC superiority, is that of Joseph and Gallagher (1980). IC animals are described as having "deficits in learning ability," coupled with a "tendency to overrespond".

More recently, however, studies using a radial maze have led Juraska et al (1984) to suggest that enrichment changes problem solving strategies and possibly even problem solving abilities. Indeed, in a separate study Einon (1980b) has suggested that differences in performance between socially housed animals and isolates "are not due to differences in spatial ability, or to differences in activity or neophobia", but that "there is some indication that the social rats may have superior memorial capacities."

In conclusion then, studies demonstrating enhanced EC performance have suggested that this can be accounted for in terms of both EC superiority in problem solving skills, or in terms of inappropriate IC learning abilities and/or cue inattention.

d)Summary of Maze Findings

Of the 90 papers reviewed in this section, 78% have found evidence of superior maze performance in animals exposed to varying degrees of environmental complexity, when compared with animals raised in isolation, or socially housed conditions. The reasons for this enhanced maze performance in the enriched animal are not clear although a variety of possible explanations have been advanced. These include a greater use of extra-maze cues (Hymovitch 1952; Forgays and Forgays 1952), an enhanced capacity to process or store information (Greenough 1972b) and possibly improved problem solving ability (Juraska et al 1984) in the enriched animal, as compared with increased exploration (Zimbardo and Montgomery 1957), and fear or reactivity (Myers and Fox 1963), coupled with a failure to habituate and a propensity towards repetitious patterns of limited and circumscribed responding (Joseph and Gallagher 1980; Dell and Rose 1986) in the deprived animal. Reid et al (1968) have also suggested that it is the relevance of the animals early experience and learning to the behavioural task which is important. Overall the evidence supports the conclusion that, as Greenough (1976) succinctly puts it "differential rearing yields behaviourally different animals and that maze performance may well reflect emotional and motivational consequences of the environments as well as differences related to the processing and

storage of information." (p 260)

2:2.2 DISCRIMINATION LEARNING

"Studies of discrimination learning involve exposing subjects to different stimuli and arranging different schedules of reinforcement for responses to each stimulus." (MacKintosh 1974 p 543) Within the EC-IC literature, there have been four types of discrimination experiments employed, involving brightness, pattern, spatial and tactile cues. The findings of each of these areas of research are summarised in Tables 2:7 and 2:8 and will be reviewed individually in the following section.

a) Brightness Discrimination

Of the 16 studies examining the effects of differential environments in a brightness discrimination paradigm, five studies have reported an EC superiority (Edwards et al 1969; Greenough et al 1972b; 1973; Bernstein 1972; 1973), one has found an SC superiority (Dawson and Hoffman 1958) and the remaining ten studies have found no differences between the groups (Bingham and Griffiths 1952; Woods et al 1960; Krech et al 1962; Gill et al 1966; Greenough 1969; Bernstein 1973; Warren et al 1972; Crnic 1983; Warren 1985; Lamden 1985). Significantly no studies have reported any evidence of IC superiority in this learning task.

Considering those studies where an enhanced discrimination performance emerged following enrichment, several methodological similarities are apparent. Firstly, in all five cases, both a perceptually and socially enriched type of environment was employed, based either on that of Hymovitch (1952), such as was used by Edwards et al (1969), or on the more common Berkeley environment of Rosenzweig and Bennett, as was used in the remaining four studies. Furthermore, although the duration of environmental experience varied between 30 and 135 days, in all instances exposure was initiated at weaning (21-25 days). In addition a two choice discrimination problem was

REFERENCE	AGE	STRAIN	SEX	COMPARISON	DISCRIMINATION APPARATUS	TYPE OF DISCRIM.
Forgus 1954	24-84	Hooded	M	CVP-MVP CV-MVP	Elevated Runway	Pattern
Nyman 1967	30-40 50-60 70-80	Hooded	M	8hrEC-SC	Alternation Maze I	Spatial
	30-40 50-60 70-80	Hooded	M	8hrEC-SC	Alternation Maze II	Spatial
Edwards et al 1969	21-90	Sprague Dawley		EC-IC	2 Choice	Brightness
Collins 1970	25-55	Mice	M	EC-IC	Curved Water T Maze	Spatial
Brown and King 1971	25-105	Sprague Dawley	M	EC-SC	Lashley Jumping Stand	Pattern
Greenough et al 1972b	25-55	Swiss Webster Mice	M	EC-SC EC-IC	2 Choice	Brightness
Bernstein 1972	21-156	Wistar	M	EC-IC	T Shaped Problem	Brightness
Greenough et al 1973	25-55	Long Evans	M	EC-SC	Operant Task	Brightness
Bernstein 1973	21-85	Wistar	M	EC-AW	T Shaped Problem	Brightness

Table 2:7 Chronological listing of all studies with an EC superiority in discrimination learning. (CVP=Complex Visual and Proprioceptive;AW=Activity Wheel; CV=Complex Visual; MVP=Minimal Visual and Proprioceptive;)

REFERENCE	AGE	STRAIN	SEX	COMPARISON	DISCRIMINATION APPARATUS	TYPE OF DISCRIMIN
Bingham and Griffiths 1952	21-51	Albino	M F	EC-IC	Lashley Jumping Stand	Brightness
Dawson and Hoffman 1958	0-50	Wistar	M F	EC-SC	Water T Maze	Brightness
Woods et al 1960	21-225	Sprague Dawley	M F	EC-IC	2 Choice Water Maze	Brightness
Krech et al 1962	21-51	S1	M	EC-IC	Krech Hypothesis Apparatus	Brightness
Gill et al 1966	21-81	Long Evans	M	EC-IC	Lashley Jumping Stand	Brightness and/or Pattern
Nyman 1967	30-40 50-60 70-80	Hooded	M	8hrEC-SC	T Maze	Pattern
Greenough 1969	no detail	no detail	no detail	EC-IC	no detail	Brightness
Finger and Fox 1971	24-60	Rattus Norvegicus	M	EC-SC	T Maze	Tactile
Bernstein 1973	21-85	Wistar	M	EC-SC	T Shaped Problem	Brightness
Finger 1978	28-60	Simonsen	M	EC-SC	T Maze	Tactile
Warren et al 1982	601-751	C57BL/6J Mice	M	EC-IC	6 Discrimination Units	Brightness
Crnic 1983	0-88	Sprague Dawley	M F	EC-SC EC-SC	Lashley Jumping Stand	Pattern Brightness
Lamden 1985	23-53	Hooded	M	EC-IC	2 Choice	Brightness
Warren 1985	no detail	Mice	no detail	EC-IC EC-IC	no detail	Brightness Spatial
Rose et al 1987	100-142	Hooded	M	EC-IC EC-SC	Bracelets	Tactile

Table 2:8 Chronological listing of all studies with no EC superiority in discrimination learning.

employed in all the experiments.

As yet, it is not clear whether this paradigm demonstrates enhanced EC learning ability per se. Indeed, Edwards et al (1969) suggest that the behavioural differences reflect differences in CNS [8] arousal, as measured by photic evoked potentials, resulting from differential rearing. Moreover, Lamden (1985) whilst reporting no significant differences between EC-IC animals' discrimination performance, noted that there were differences in aspects of the overall performance of the two groups as the brightness differential was decreased. In particular, IC animals took longer to make the discrimination with decreasing brightness differences. This may well reflect underlying perceptual differences between the groups. Indeed, Lamden suggests that, although not translated into incorrect discrimination responses, IC animals may require more time to process sensory information.

With respect to those studies reporting no differences between the groups, certain important methodological problems must be considered. As Krech et al (1962) point out, the early studies (Bingham and Griffiths 1952; Dawson and Hoffman 1958; Woods et al 1960) neglected certain important precautions, thus rendering their results equivocal. These include the prejudicing of results by the experimenter being fully aware which animal came from which environment, coupled with the possibility of "confusing increased learning ability with the effects of specific positive transfer to handling" (p 801-802) and "the confounding of exploratory behaviour with error scores" (p 802). Furthermore, both Dawson and Hoffman (1958) and Woods et al (1960) employed water mazes in which the response requirements were very different from the usual motor requirements of a runway situation. Indeed as Lamden (1985) points out, it is possible that the response requirements were so unfamiliar to all the subjects, that discrimination differences may have been submerged in the motor learning component.

With respect to the "later" studies, other methodological differences must also be taken into account. Firstly, certain features of the 1962 study by Krech et al may well render comparison

[8] Central Nervous System

with the other studies untenable. The Krechevsky Hypothesis Box is typically employed for *reversal* discrimination learning, and is generally not used for brightness discrimination acquisition per se. The apparatus consists of four successive two-choice discrimination boxes, at each choice point the animal being confronted with a lightened and darkened alley, only one of which leads to the next unit or goal box. Animals are given ten trials daily (comprising 40 discriminations) and are trained to a criterion of 19/20 consecutive correct choices. In comparison with the other studies in this area of research, this paradigm requires the animals to make more than one discrimination to successfully traverse the apparatus. Consequently, this procedure can be regarded as fundamentally different and its results should be treated separately from the other findings in this literature that have used two-choice discrimination paradigms.

Secondly, as well as employing a multiple-choice discrimination unit, Warren et al (1982) also introduced another variable into their design, namely age of onset of differential experience. In this study, mice were housed in standard colony conditions (four to five per cage) until 601 days old, and then placed into differential environments. In an earlier study, Bernstein (1972) reported that only when exposure to the enriched environment exceeded the time spent in an earlier and more restricted environment would the effects of enrichment be manifested in a discrimination apparatus. This was not the case in the Warren et al study. Age of onset was also unusual in the only study where EC animals were found to be *inferior* to another group (Dawson and Hoffman 1958). In this instance, animals were exposed to either enriched or standard environments from birth and were trained on a brightness discrimination task at 40 days old. Interestingly, initial learning scores of the two groups, although statistically insignificant, suggested a definite trend in favour of the EC group. However, when tested on a re-learning paradigm, SC animals were significantly superior to EC animals. Dawson and Hoffman (1958) attribute this to retroactive inhibition, in that increased activity such as would be experienced by EC animals during the interpolated period between original learning and relearning, inhibited relearning.

In summary, the majority of studies examining the effects on brightness discrimination in animals

exposed to differential environments have found no significant differences between the groups. However, of these studies, two (Krech et al 1962; Warren et al 1982) have used complex multiple-choice discrimination procedures, which render comparison with the remaining studies difficult. Furthermore, of the remaining nine studies, three (Bingham and Griffiths 1952; Dawson and Hoffman 1958; Woods et al, 1960) have been criticised for poor experimental methodology, making interpretation of their data problematic. As to the five studies finding significant differences between differentially housed animals, it is still unclear whether enhanced EC performance reflects improved learning ability in the EC animal or differences in perceptual abilities (Lamden 1985) or arousal levels (Edwards et al 1969) between the groups.

b) Pattern Discrimination

The ameliorative effects of early *visual* experience on pattern discrimination is well documented (Forgus 1956; McCall and Lester 1969; Lavallee 1970; Corrigan and Carpenter 1979). The effects of a *socially and perceptually* enriched environment, however, are less clear cut. Of the five studies examining the pattern discrimination performance of animals exposed to differential environments, only two have found evidence of EC superiority in this task (Forgus 1954; Brown and King 1971). Of the remaining studies, two have reported no differences between the groups, (Gill et al 1966; Nyman 1967) and one has described an SC superiority in pattern discrimination (Crnic 1983).

Examination of the procedures involved in these studies, reveals a wide variation in methodological detail. Firstly, with respect to environmental manipulation, Forgus (1954) found that animals exposed to both complex visual and proprioceptive (CVP) experience to be superior performers when compared with animals housed in a minimal visual and proprioceptive environment (MVP). However, animals exposed to complex visual experience (CV) alone were significantly better performers than the other two groups (CVP or MVP). The latter finding was attributed to the fact that for the CV group, visual stimuli in the discrimination apparatus were more promi-

nent, whereas for the CVP group, kinesthetic cues were also perceptually prominent, and needed to be eliminated prior to completing the form discrimination test. This was later confirmed (Forgus 1955a) when, unlike the previous paradigm, where the stimulating field contained many irrelevant extra-visual stimuli as well as the relevant visual cues, the apparatus was best solved by only utilising visual cues. In this instance, no significant differences were found between the CV and CVP groups. The effects of manipulation of both amount and variety of stimulation and the addition of formal training to enriched experience on pattern discrimination have also been investigated (Brown and King 1971). Although significantly different from the SC groups, only those enriched groups receiving variety of stimulation were significantly improved in their discrimination performance, when compared with all the enriched groups' performances. Indeed, as Brown and King (1971) point out, formal training and absolute levels of stimulation appear to be relatively unimportant for behavioural changes following environmental enrichment. Interestingly, the studies finding no evidence of EC superiority in a pattern discrimination task, used enriched environments similar to those recommended by Rosenzweig and Bennett (1977), although in one case (Crnic 1983) handling was also included as a form of experiential enrichment. Furthermore, of the five studies, only one (Gill et al 1966) compared enrichment with impoverishment. The remaining studies employed a standard condition containing either two (Crnic 1983), four (Nyman 1967; Brown and King 1971) or 14 (Forgus 1954) animals.

Secondly, age of onset of differential experience and length of experience have been varied. Both studies finding significant differences between the groups (Forgus 1954; Brown and King 1971) in favour of EC animals exposed animals to environments at weaning, for 60-80 days, as did one of the studies finding equivalent performance between the groups (Gill et al 1966). Nyman (1967) however, exposed animals for either one or eight hours a day, for ten days, starting at 30, 50 or 70 days of age, whereas the subjects used in by Crnic (1983) were born into differential environments.

Thirdly, variation also exists in the procedures employed. Three types of apparatus have been

used, including an elevated runway (Forgus 1954) a T maze (Nyman 1967) and the Lashley Jumping Stand (Gill et al 1966; Brown and King 1971; Crnic 1983). However, even those investigators using the same apparatus (Lashley Jumping Stand) have varied the procedure to such an extent that it is difficult to determine whether strictly comparable behaviours were being assessed in each situation. For example, Gill et al (1966) deprived their animals to 80% of their free feeding weight and then used shock as the motivator in the test situation, whereas Brown and King (1971) although using shock, fed their animals on an ad libitum schedule. Crnic (1983) however, used non-deprived animals and simply pushed them if they failed to respond to the discrimination within a specified time interval. As Lamden (1985) points out, the motivational states of the animals must surely differ considerably between these studies.

Overall, therefore, it is difficult to assess the effects of differential environments on pattern discrimination learning, given the variation in the methodologies employed.

c) Spatial discrimination

To date, only three studies have examined the effects of differential environments on spatial discrimination (Nyman 1967; Collins 1970; Warren 1985), of which two (Nyman 1967; Collins 1970) have found EC animals to be superior performers, the third reporting no differences between the groups. These results appear to interact with the age of onset of differential experience, and the number of hours spent in the environments (Nyman 1967). In particular, animals exposed to enrichment for eight hours a day were superior performers to animals exposed to enrichment for one hour a day. Futhermore, exposure between 50-60 days was more effective than exposure between either 30-40 or 70-80 days.

As with previous studies examining the effects of EC on discrimination performance, there are also large methodological differences between the work of Nyman and Collins [9]. Nyman tested his *rats* on an alternation maze using a food reward, whereas Collins measured discrimination

[9] Unfortunately few methodological details were available for the Warren paper, rendering comparison difficult.

performance in *mice* in a curved T maze filled with water, escape providing the reinforcement. Futhermore, Collins housed her "impoverished" group in isolation, whilst Nyman's animals were housed in groups of three or four.

Indeed, given the paucity of studies in this area, caution must be used in interpreting these findings as demonstrating EC superiority in spatial discrimination. However, it must also be noted that the evidence from the maze studies (reviewed earlier in this chapter), the methodology of which incorporates a large element of spatial ability, would tend to support the present findings of improved spatial ability in the EC animal.

d) Tactile Discrimination

To date, three studies have looked at the effects of differential environments on tactile discrimination performance (Finger and Fox 1971; Finger 1978; Rose et al 1987) and in none of the studies have any significant differences emerged between the groups. As with previous research, idiosyncratic methodologies render comparison difficult and generalisation of these results must be undertaken with caution. However, two similarities exist between the studies, namely all employed surgery and investigated the therapeutic effects of enrichment on performance.

In Finger's studies, animals were bilaterally enucleated at weaning and then housed in groups of three or four for approximately 34 days, during which time half the animals were exposed to a range of fixed and movable objects of different texture. This enriched condition thus offered both spatial complexity and a range of tactile experience. In the first study (Finger and Fox 1971) animals were tested on five different discriminations, of varying complexity, immediately after the differential experiences. In the second study (Finger 1978), however, approximately half the animals received large bilateral lesions of the two somatosensory areas of the cortex, the remaining animals undergoing sham operations. After a recovery period, testing was initiated in the T maze employed in the earlier experiment. No significant differences were found between the EC-SC groups in either experiment, although some of the tasks were found to be harder to

discriminate than others (Finger and Fox 1971). Similarities in performance between the groups were attributed to the spatial rather than the tactile features of the test, both blinded groups having developed competence in making spatial adjustments within their home cages.

The study of Rose et al (1987) also merits some discussion, as it employs an unusual form of tactile discrimination task. Based on reports in the human neurological literature (Friedland and Weinstein 1977) that there is a degree of neglect of stimuli presented to the side of the body contralateral to large neocortical lesions, Rose et al (1987) investigated the possible therapeutic effects of environmental enrichment on this phenomenon, using an animal model of contralateral neglect developed by Schallert and Whishaw (1984). The discrimination task involved timing the contact and removal of simultaneously positioned paper bracelets from the forepaws ipsilateral and contralateral to a large unilateral lesion, discrimination in this instance involving a decision concerning the presence or absence of a tactile cue on the arm contralateral to said lesion. No differences in latency to remove the contalateral bracelets were found between animals housed post operatively in either EC, IC or SC conditions, in either the lesioned or sham groups. There was, however, significant contralateral neglect in the lesioned animals. Rose et al (1987) suggest that their results are consistent with the view that environmental enrichment does not aid recovery of function per se, and that earlier findings of ameliorative and therapeutic effects of enrichment (Rose 1987) can best be explained in terms of compensation rather than recovery.

In summary, it appears from the above results that there are no significant differences between differentially housed animals in tactile discrimination. However, as with the spatial discrimination literature, the paucity of the studies makes firm conclusions premature.

e) Summary of Discrimination Findings

Of the 27 experiments in this section, only 37% have found evidence of EC superiority in discrimination learning. However, this percentage is not proportionally represented across the four categories of discrimination learning reviewed above. Enrichment is particularily effective in

improving performance in spatially based tasks, as demonstrated by the fact that two of the three studies in this paradigm show EC performance superiority. Enrichment also influences both visual discrimination paradigms, with 40% of all the pattern and 29% of all the brightness discrimination studies demonstrating enhanced performance in the EC animal. With respect to tactile discrimination learning, however, no significant differences were found between differentially housed groups in any of the three studies in this category. In none of the studies was there an IC superiority effect, although two studies found evidence of improved SC performance when compared with EC animals (Dawson and Hoffman 1958; Crnic 1983).

As yet it is not clear whether enrichment enhances learning *per se* in this type of learning paradigm, or whether the observed behavioural differences merely reflect differential arousal levels (Edwards et al 1969) or underlying perceptual abilities (Lamden 1985). Indeed, the variety of methodological procedures makes any firm conclusions difficult. However, the pattern of differences that has emerged suggests that the visual and spatial abilities of enriched animals are more appropriate for the tasks outlined above than are those of their IC counterparts. Furthermore, in a recent study Van Woerden (1986) [10] has offered evidence that the relative novelty of the cue stimulus affects EC and IC animals differently. Typically, in discrimination tasks where the animals were habituated to the cues by being exposed to them prior to testing, EC rats performed better than their IC counterparts. If, however, the cues were novel, then no differences between the groups were found. This has implications for the importance of the EC experience in attenuating adaption in these animals and has consequences for the methodologies employed in this type of research. Furthermore, Van Woerden's work does appear to support Lamden's (1985) idea that the cues employed in this task have differential meaning for the groups, although she (Lamden) believes this is more to do with perceptual abilities than novelty.

[10] This study was reported in Rosenzweig and Renner (1987) and forms part of an unpublished doctoral dissertation. As few details were available it is not included in any of the tables.

2:2.3 DISCRIMINATION REVERSAL LEARNING

"Reversal training involves reversing the reinforcement contingencies in effect during an initial discrimination problem" (MacKintosh 1974 p601) and as a paradigm has frequently been adopted in learning research, as it is assumed to represent a more complex form of instrumental learning. Within the EC-SC-IC literature, there have been 23 experiments of reversal learning (see tables 2:9 and 2:10) employing a wide range of discriminations, of which seven have found significant evidence of EC superiority, when compared with IC animals (Krech et al 1962; Klein 1968; Bennett et al 1970 (two studies); Collins 1970; Morgan 1973 (two studies)) and one of EC superiority when compared with SC animals (Doty 1972). In addition, two studies have found evidence of SC superiority when compared with IC animals (Bennett et al 1970; Einon et al 1978) and one has found evidence of IC superiority, when compared with EC littermates (Bennett et al 1970). The remaining 12 studies have reported no significant differences between the groups employed, although, in three studies (Bennett et al 1970) the tendency was for the EC animals' performance to be better than that of the IC groups.

The first report of EC superiority in reversal discrimination learning (Krech et al 1962) employed the Krech Hypothesis Apparatus, which as detailed previously, consists of four successive units of two-choice discrimination boxes. The subjects in this experiment were exposed at weaning to 30 days of environmental enrichment or impoverishment and were subsequently trained on a light-correct brightness discrimination task, until a criterion of not more than one error in five successive trials (in this instance 19 correct choices out of 20) was reached, at which time the first reversal problem, dark-correct, was introduced. When this was mastered, the animals were required to relearn the light-correct discrimination. This sequence of discrimination learning and reversal was continued for 18 days. In a later experiment, Bennett et al (1970) using an identical apparatus, replicated Krech et al's earlier findings, as did Klein (1968). In the latter study, however, a different strain of rat was employed. Collins (1970) and Morgan (1973) also found similar results in their studies, despite the variations in their methodologies in terms of strain

REFERENCE	AGE	STRAIN	SEX	COMPARISON	APPARATUS	TYPE OF DISCRIM
Krech et al 1962	25-55	S1	M	EC-IC	Krech Hypothesis	Brightness
Klein 1968	25-55	S3	M	EC-IC	Krech Hypothesis	Brightness
Bennett et al 1970	25-55	S1	M	EC-IC	Krech Hypothesis	Brightness
	25-185	S1	M	EC-IC	Krech Hypothesis	Brightness
	25-55	S1	M	SC-IC	Krech Hypothesis	Brightness
Collins 1970	25-55	Mice	M	EC-IC	Curved T Water Maze	Spatial
Doty 1972	300-660	Sprague Dawley	M F	EC-SC	Shuttle-box	Brightness
Morgan 1973	20-80	Hooded	M	EC-IC	Push/Pull Task	
	20-110	Hooded	F	EC-IC	Push/Pull Task	
Einon et al 1978	23-105	Hooded	F	SC-IC	Push/Pull Task	

Table 2:9 Chronological listing of all studies with an EC or SC superiority in reversal learning. (NB: Klein (1968) cited in Bennett et al 1970)

REFERENCE	AGE	STRAIN	SEX	COMPARISON	APPARATUS	TYPE OF DISCRIM
Dawson and Hoffman 1958	0-30	Wistar	M F	EC-SC	Water T Maze	Brightness
Gill et al 1966	21-81	Long Evans	M	EC-IC	Lashley Jumping Stand	Pattern
Bennett et al 1970	25-105	S1	M	EC-IC (a)	Krech Hypothesis	Brightness
	60-90	S1	M	EC-IC (b)	Krech Hypothsis	Brightness
	90-120	S1	M	EC-IC (c)	Krech Hypothesis	Brightness
	60-120	S1	M	EC-IC (a)	Krech Hypothesis	Brightness
	25-85	S1	M	EC-IC (a)	Krech Hypothesis	Brightness
	25-85	S1	M	SC-IC (d)	Krech Hypothesis	Brightness
Finger and Fox 1971	24-60	Rattus Norvegicus	M	EC-SC	T Maze	Tactile
Morgan 1973	21-75	Hooded	M F	EC-SC	Push/Pull Task	
	21-61	Hooded	M F	EC-SC	Push/Pull Task	
Warren 1985	no detail	Mice	no detail	EC-IC	no detail	Brightness
		Mice		EC-IC	no detail	Spatial

Table 2:10 Chronological listing of all studies with no EC superiority in reversal learning.
(a)=Tendency for EC>IC, but not significant.
(b)=IC significantly superior to EC.
(c)=Tendency for IC>EC, but not significant.
(d)=Tendency for SC>IC, but not significant.

of rat, type of discrimination and type of apparatus. Indeed, Collins measured discrimination performance in a curved T maze filled with water (Waller, Waller and Brewster 1960), with escape from the water via a ladder following a correct discrimination. Morgan (1973), on the other hand, trained his animals to remove an obstacle from an alleyway, either by pushing it forwards, or pulling it backwards, in order to access a food compartment. After being trained to push or to pull, rats were confronted with a transfer problem in which the direction was reversed. Interestingly, the transfer from pushing to pulling tended to be easier than that from pulling to pushing. Although the EC group's performance was superior to that of the IC group, Morgan points out that to suggest that isolates are simply less "intelligent" than group reared animals is too vague to be useful. A speculative hypothesis, he suggests, is that "rats reared in isolation have a reduced capacity for behavioural inhibition. The most striking fact about their behaviour is their slowness in abandoning a previously successful strategy" (p440), which was later supported by Dell and Rose (1986) in a Hebb-Williams procedure, where IC's demonstrated an inadequate response inhibition.

In all the studies reported above, differential experience was initiated at weaning (20-25 days). Doty (1970), however, compared EC animals with animals housed in same-sex pairs (SC), with differential experience starting at 300 days and lasting for 360 days. Her animals were required to learn a discriminated light-dark reversal in a two-way shuttle box and, again, EC animals were found to be superior performers. Futhermore, both Einon et al (1978) and Bennett et al (1970) have found SC groups' performance to exceed that of their IC counterparts.

With respect to the studies described in Table 2:10 in which no EC superiority has been found, there is clearly no one identifiable factor (strain of rat, environmental manipulation, type of apparatus or type of discrimination) which clearly distinguishes these studies from those reporting EC superiority, as outlined above and in Table 2:9. Nor is the answer to be found in the scoring methods, since these were necessarily varied due to the different types of responses required by specific test situations (Lamden 1985). Dawson and Hoffman (1958), for example, measured

latency to escape from a single unit water T-maze, which was only possible after correct reversals of the light-dark discrimination. In this experiment, differential experience was initiated at birth and the control group consisted of socially housed animals. SC groups have also been used by both Finger and Fox (1971) and Morgan (1973) with tactile and motor discriminations being employed respectively; in both studies, no EC superiority in performance was observed. Gill, Reid and Porter (1966) and Bennett et al (1970) have employed *EC-IC* comparisons. Gill et al (1966) measured latency to leave the platform of a Lashley Jumping Stand in animals exposed to EC-IC conditions for 60 days following weaning. Interestingly, training on a reversal discrimination in this particular apparatus produced stereotypic responding, described by Maier (1949) as "frustration fixation" in all the subjects and despite retraining the animals' results were poor. Finally, Bennett et al (1970) recorded the number of reversals correctly learned by at least half the members of a particular group of animals, enriched animals typically learning more reversals successfully than their impoverished counterparts.

Of all the possible sources of variation which may have contributed to the non-significance of the results of the studies in Table 2:10, one factor which has been sytematically varied by the latter experimenters in particular, namely age at which environmental experience was initiated, seems to merit further consideration. Indeed, both Krech et al (1962) and Bennett et al (1970) maintain that enriched experience results in superior reversal discrimination performance, *only* when it is given immediately after weaning. Considering Tables 2:9 and 2:10, in nearly all of the experiments in which animals were put into EC-IC at about 25 days of age, the expectation of superior performance on the part of the EC animal appears to be justified. As Bennett et al (1970) point out, "EC animals in general solved significantly more problems and made significantly fewer errors per reversal" (p72) than did their IC counterparts. Furthermore, they report that in their two experiments where no EC-IC differences were observed with differential experience initiated at weaning, there was still a tendency for the EC group to be superior to the IC group.

The contention that experience starting at weaning is a prerequisite for EC performance superiority is further substantiated by the evidence obtained from animals introduced into the EC or IC conditions at either 60 or 90 days of age (Bennett et al 1970). In particular, enrichment from 60-90 days produced animals which solved *fewer* problems and made *more* errors per reversal problem than did their IC littermates. No significant differences emerged in the 60-120 day experiment, but for the 90-120 day experiment EC animals again tended to solve fewer problems than the IC animals. Indeed, these data, according to Bennett et al (1970) "give a striking indication of a critical period for beneficial behavioural effects of environmental enrichment" (p72). Preweaning EC, even when it was extended to 40 days of age (Dawson and Hoffman 1958) did not enhance reversal learning ability either, although it must be noted that in this experiment EC animals were compared with SC controls rather than isolates.

It appears that start age, therefore, has an effect on EC-IC performance, although caution must be exercised in interpreting these results in an absolute manner, as Gill, Reid and Porter (1966) reported that their animals did *not* differ in reversal discrimination performance, even when the environmental experience began at *21 days*. However, it must also be pointed out that in their experiment the Lashley Jumping Stand was employed, a piece of apparatus which they observed to be "difficult and frustrating" (p240).

Duration of experience, unlike start age, does not appear to be an important variable. Indeed, when animals were put into EC or IC at 25 days of age (Bennett et al 1970), the difference in "problem solving ability" in favour of the EC animals could not be distinguished according to whether the animals remained in the experimental conditions for 30, 60, 80 or 160 days. In this connection, it might be recalled that one of the brain weight measures, namely the ratio of weight of cortex to that of the rest of the brain, also showed EC-IC differences that were significant and of about the same magnitude regardless of length of treatment, when this started at 25 days of age.

The difference between those experiments in which environmental experience is initiated at wean-

ing and those in which the starting age is moved from 25 days to 60 or 90 days, may be partially explained when the results of the SC animals are considered (Bennett et al 1970). Typically, in the Berkeley studies all animals were held in standard colony conditions (three animals to a cage) prior to EC or IC beginning at 60 days of age. In the visual discrimination reversal task SC animals have been found to be superior to IC animals (Bennett et al 1970; Einon et al 1978) and equivalent to EC animals (Dawson and Hoffman 1958; Finger and Fox 1971; Morgan 1973). Thus it would appear that performance can be impaired if an animal is placed in *isolation* but that this reversal task is not sensitive to enrichment over and above the colony experience. Indeed, the behavioural results of the Krech Hypothesis test indicate that the difference between the EC and IC animals should be attributed to early restriction of experience and not to enrichment of experience. Consequently, when IC is begun at 60 days, it is *not* effective, as once an animal has been in the colony condition it is protected against the deleterious effects of isolation.

Finally, the one study reporting superior IC performance (Bennett et al 1970) differs from the studies yielding superior EC and SC performance only in that environmental experience was given from days 60-90. As Lamden (1985) points out, "no explanation has been offered for this result" (p85).

In summary, it appears that differences in discrimination reversal training between EC, SC and IC groups' performances can be attributed to the deleterious effects of restricted experience. Indeed Morgan (1973) has suggested that IC animals have a reduced capacity for response inhibition, which produces inappropriate behaviours in this type of learning paradigm.

2:2.4 AVOIDANCE LEARNING

Avoidance learning relies on an animal responding in such a way as to prevent the occurrence of an aversive event. Within the EC-SC-IC literature, two avoidance learning paradigms have been employed, namely *active avoidance* in which an active response prevents the onset of an aversive stimulus and *passive avoidance*, in which inhibiting movement prevents the onset of an aversive

stimulus. Interestingly, in rats, with shock as the negative reinforcer, it is the latter paradigm that is learned more readily (Brener and Goesling 1970). In the following section the effects of differential environments on performance in active and passive avoidance tasks will be reviewed.

a) Active Avoidance Learning

Of the 12 experiments examining the effects of differential environments on active avoidance learning (see Tables 2:11 and 2:12), only three have found evidence of EC superiority (Ray and Hochhauser 1969; Freeman and Ray 1972; Joseph and Gallagher 1980). Of the remaining nine experiments, four have found evidence of IC superiority (Lovely et al 1972; Parsons and Spear 1972-2 experiments each), one has found evidence of SC superiority when compared with EC (Freeman and Ray 1972) and four have found no significant differences between the groups (Doty 1972; Freeman and Ray 1972-two experiments; Ferchmin et al 1980). Considering Tables 2:11 and 2:12, it is not immediately obvious that any one factor is responsible for the diversity of the results. Indeed, in the active avoidance learning literature, unlike other learning paradigms reviewed earlier in this chapter, a fairly standardised form of testing has been employed, with all but one of the studies (Joseph and Gallagher 1980) using a shuttle box apparatus.

Ray and Hochhauser (1969), Freeman and Ray (1972) and Joseph and Gallagher (1980), who reported enhanced active avoidance learning following EC experience, made use of similar experimental conditions and all kept their animals in differential environments from weaning for 53-64 days. However, whilst Joseph and Gallagher (1980) housed their IC group in individual cages, both Ray and Hochhauser (1969) and Freeman and Ray (1972) maintained their "isolated" groups in pairs. Interestingly, Joseph and Gallagher have suggested that it is the housing of an animal in a restricted environment that leads to a "rearing-dependant deficit in learning, and the selective directing of responses to adaptive ends" (p541). However, whether restriction *per se* is responsible for these results is called into question by the findings of Loveley et al (1972) and Parsons and Spear (1972). In the former paper, both 30 and 50 days of group housing versus

REFERENCE	AGE	STRAIN	SEX	COMPARISON	APPARATUS	PROCEDURAL DETAIL
Ray and Hochhauser 1969	21-85	Charles River	M F	EC-SC (pairs)	Shuttle Box	150 trials
Freeman and Ray 1972	28-88	Zivic Miller	M F	EC-SC (pairs)	Shuttle Box	125 trials
Joseph and Gallagher 1980	19-72	Zivic Miller	M F	EC-IC	Step-Up To Perch	latency to step

Table 2:11 Chronological listing of all studies with an EC superiority in Active Avoidance Learning.

REFERENCE	AGE	STRAIN	SEX	COMPARISON	APPARATUS	PROCEDURAL DETAIL
Lovely et al 1972	105-135	Long Evans	M	SC-IC	Shuttle Box	100 Acquisition Trials 100 Extinction Trials
	105-155	Long Evans	M	SC-IC	Shuttle Box	100 Acquisition Trials 100 Extinction Trials
Parsons and Spear 1972	23-83	Sprague Dawley	F	EC-IC	Shuttle Box	5 Trials
	80-160 or 110-190	Sprague Dawley	F	EC-IC	Shuttle Box	Trials to Relearn
Doty 1972	300-660	Sprague Dawley	M F	EC-SC (pairs)	Shuttle Box	Trials to Criterion
Freeman and Ray 1972	28-88	F344 /FMai	M F	EC-SC (pairs)	Shuttle Box	125 trials
	28-55	Zivic Miller	M F	EC-SC (pairs)	Shuttle Box	125 Trials
	28-55	F344 /FMai	M F	EC-SC (pairs)	Shuttle Box	125 Trials
Ferchmin et al 1980	30-60	Rattus Norvegicus	M	EC-IC	Shuttle Box	25 Trials

Table 2:12 Chronological listing of all studies with no EC superiority in Active Avoidance Learning.

isolation, produced individually housed animals that were facilitated in both the acquisition and extinction of a shuttle box conditioned avoidance response. In the latter paper, Parsons and Spear (1972) showed that rats which spent their retention interval in an enriched environment showed greater forgetting of active avoidance, as measured by trials to relearn, when compared with animals housed in individual cages. In this study, however, no evidence of IC superiority was found in the acquisition phase of the experiment. The only discernable difference between the methodologies employed by these investigators and those used by Ray and Hochhauser (1969), Freeman and Ray (1972) and Joseph and Gallagher (1980) is the choice of dependant variable.

Other than choice of dependant variable, another explanation of the source of variation in experimental findings has been offered by Freeman and Ray (1972). They reported an interaction between rat strain and rearing complexity. However, the 12 studies of active avoidance learning have used a variety of animal strains and the use of a particular strain does not appear to guarantee the results (Lamden 1985). Overall, with respect to the effects of differential experience on active avoidance, to date no clear pattern has emerged and more rigorous examination of strain and task specificity is required before any firm conclusions can be drawn.

b) Passive Avoidance Learning

As can be seen from Table 2:13, six studies have found evidence of superior EC performance on a passive avoidance task, of which five compared EC animals with IC animals (Lore 1969; Greenough et al 1970; Gardner et al 1975; Domjan et al 1977; Joseph and Gallagher 1980) and one with SC animals (Doty 1972). Table 2:14 describes those studies where no EC performance superiority has emerged. Of the 11 studies detailed, five have found SC animals to be superior to EC animals (Freeman and Ray 1972, four studies; Crnic 1983) and one has found EC/SC equivalence (Gardner et al 1975), four have found no differences between EC/IC groups (Gibson et al 1968; Kirkby 1970; Parsons and Spear 1972; Davenport 1976) and one has found SC animals to be superior to IC animals. In none of the studies has an IC performance been superior to

REFERENCE	AGE	STRAIN	SEX	COMPARISON	APPARATUS	PROCEDURAL DETAIL
Lore 1969	21-67	Long Evans	M F	EC-IC	Candle	2 Trials
Greenough et al 1970	25-81	DBA2J Mice	M	EC-IC	Step-Down	2 Trials
Doty 1972	300-660	Sprague Dawley	M F	EC-SC	Step-Thro'	Trials to Criterion
Gardner et al 1975	21-82 or 21-93	Long Evans	M	EC-IC	Step Down	2 Trials
Domjan et al 1977	21-60	Sprague Dawley	M F	EC-IC	Step Down	Trials to Criterion
Joseph and Gallagher 1980	19-72	Zivic Miller	M F	EC-IC	Step Down	Latency To Step Down

Table 2:13 Chronological listing of all studies with an EC superiority in Passive Avoidance Learning.

REFERENCE	AGE	STRAIN	SEX	COMPARISON	APPARATUS	PROCEDURAL DETAIL
Gibson et al 1968	21-81	Long Evans	M	EC-IC	Step Down	10 Trials
Kirkby 1970	20-50	Sprague Dawley	M F	EC-IC	Step Down	3 Trials
Parsons and Spear 1972	23-83	Sprague Dawley	F	EC-IC	Step Thro'	Latency
Freeman and Ray 1972	28-88	Zivic Miller	M F	EC-SC (pairs)	V Shaped Box	2 Trials
	28-88	F344 /FMai	M F	EC-SC (pairs)	V Shaped Box	2 Trials
	28-55	Zivic Miller	M F	EC-SC (pairs)	V Shaped Box	2 Trials
	28-55	F344 /FMai	M F	EC-SC (pairs)	V Shaped Box	2 Trials
Gardner et al 1975	21-82 or 21-93	Long Evans	M	SC-IC EC-SC	Step Down	2 Trials
Davenport 1976	36-70	Holtzman	M F	SEC-IC	Step Down	3 Trials
Crnic 1983	25-55	Sprague Dawley	M F	EC-SC (pairs)	Step Thro'	2 Trials

Table 2:14 Chronological listing of all studies with no EC superiority in Passive Avoidance Learning.

either EC or SC groups.

Once again no single factor clearly differentiates these studies. Indeed, environmental conditions seem comparable, as do testing environment and choice of dependant variable. However, one possible explanation for the wide variation in results has been suggested by the work of Gardner et al (1975), in which both the perceptual and social aspects of environmental stimulation were manipulated. Prolonged exposure to socially and perceptually *impoverished* environments was found to significantly impede passive avoidance learning. In a second experiment, the question of the possible mediatory involvement of memory processes in this impoverished deficit was addressed. An amnesic agent (electroconvulsive shock) was employed and it was found that socially impoverished animals (be they perceptually enriched or not) were as affected by the amnesic agent at 60 seconds after training as at 10 seconds after training. This suggested a slowing of the consolidation processes and futhermore, of particular interest to the present discussion, these effects seemed primarily the result of *social* rather than perceptual impoverishment during rearing. Indeed, it may be that the better passive avoidance learning of socially enriched animals (be they perceptually enriched or not) can be explained in terms of experientially specific transfer. As Gardner et al (1975) point out "socially enriched rats growing up and living together provide each other with noxious as well as rewarding stimuli, and provide opportunities for passive avoidance situations" (p326). Indeed, they speculate that "the behavioural effects of social and perceptual environments may be qualitatively different, and may possibly be mediated by seperate physiological mechanisms" (p326).

Indeed, considering the 16 studies in both Tables 2:13 and 2:14, only four (Gibson et al 1968; Kirkby 1970; Parsons and Spear 1970; Davenport 1976) have found no significant differences between socially (and in this instance perceptually) enriched and socially impoverished animals. A point to emphasise is that in all the other studies, either EC or SC groups have been superior performers when compared with IC groups. However, there is still one question which remains unresolved, namely is social enrichment alone more efficacious than social and perceptual enrich-

ment combined? Examination of the data as yet does not yeild any conclusive results. Doty (1972) for example, found her EC animals to be significantly better performers than her SC animals, although a late start age (300 days) may well have contributed to this finding. On the other hand, Freeman and Ray (1972) and Crnic (1983) have both found SC animals to be better performers than their EC counterparts, whereas Gardner et al (1975) found no significant differences between the groups. What is clear however, is that a degree of social enrichment is beneficial to an animal's performance on a passive avoidance task.

In conclusion, the pattern that is emerging in the passive avoidance literature, suggests that a degree of social experience is beneficial to an animal, in that it provides experience of avoiding noxious stimuli which can be transfered to the testing situation. Whether this is the whole explanation of the results reported above is still unclear.

c) Summary of Avoidance Learning Findings

Within the EC-SC-IC literature, there have been 28 studies examining avoidance learning, of which a third (32%) have found evidence of EC superiority. With respect to active avoidance, behaviour seems to be determined more by strain and task, than by cognitive capacity per se, although strain and cognitive capacity might well be linked (Cooper and Zubek 1958). With passive avoidance, however, a degree of social enrichment appears to contribute to improved performance. As yet however, no firm conclusions can be drawn as to the benefits of a complex environment in improving performance in an aversive reinforcement situation. Indeed, the interpretation of EC-IC differences on tasks mediated by presentation of exteroceptive stimuli has been opened to question, by the report of Rose, Love and Dell (1986), who found that the relationship between the brightness of a barpress-contingent light and its effect on barpressing differs for EC and IC rats. Brief presentations of light become aversive at lower intensity for EC than for IC rats. If, in fact, the same physical stimulus carries a different significance for IC than for EC subjects, as is implied by these results, there are important implications for

changes brought about by environmental differences. As Renner and Rosenzweig (1987) point out "behavioural differences discovered through the use of tasks involving punishment (passive and active avoidance) may have to be reinterpreted, as footshock of a particular intensity may be perceived as differently aversive by the two groups and their subsequent performance could not then be clearly ascribed to differences in information processing or behavioural abilities"(p47). This interpretation is supported by the reports of Woods et al (1961) that EC and IC respond differently to manipulation of level of food deprivation [11] and of Juraska et al (1983) that IC rats show lower convulsive thresholds than EC. Consequently, care must be taken in drawing any firm conclusions from the performances of differentially reared animals in avoidance learning tasks.

2:2.5 SKINNER BOX CONDITIONING

Within the EC-IC literature there have been 19 studies that have employed an operant conditioning paradigm, of which 14 have looked at the acquisition and extinction of bar press rates using simple fixed ratio or variable interval schedules of reinforcement. The remaining five studies have employed more complex paradigms, in particular the differential reinforcement of low rates of responding (DRL), and GO-NO-GO procedures. The findings of each of these areas will be reviewed separately in the following pages.

a) Simple Operant Procedures

Table 2:15 lists chronologically the studies that have employed simple operant conditioning paradigms. One aspect of the results of these studies which is particularily interesting concerns the data obtained from the *acquisition* phase of the experiments. Using simple reinforcement schedules (CRF, V.I. or FR2), in eight out of the 14 studies IC animals have been found to bar press *more* than their EC counterparts (Coburn and Tarte 1976; Lamden and Rose 1979; Joseph and Gallagher 1980; Nau, Elias and Bell 1981; Rose and Lamden 1983; Rose, Dell and Love

[11] IC animals are more food oriented when hungry than their EC counterparts.

REFERENCE	TREATMENT AGE	STRAIN	GENDER	COMPARISON
Gibson, Gill and Porter 1968	21-81	Long Evans	M	EC-IC
Ough, Beatty and Khalili 1972	21-70	Holtzman	M F	EC-IC
Davenport 1976	36-70	Holtzman	M F	SEC-IC
Coburn and Tarte 1976	21-60	Wistar	M F	EC-IC
Gluck and Pearce 1977	21-111	Long Evans	M F	SC-IC
Will, Ungerer, Pallaud and Ropartz 1977	25-85	Fischer or August	M F	EC-SC
Lamden and Rose 1979	21-81	Hooded Lister	M	EC-IC
Joseph and Gallagher 1980	19-72	Zivic Miller	M F	EC-IC
Freedman and Villeneuve 1981	25-85	Hooded Rats	M	EC-IC
Nau, Elias and Bell 1981	0-101	Fischer		EC-SC-IC Handling
Rose and Lamden 1983	21-51	Hooded Lister	M	EC-IC
Rose, Dell and Love 1985a	21-51	Hooded Lister	M	SEC-EC-SC-IC-IHC
Rose, Love and Dell 1986	21-51	Hooded Lister	M	EC-IC
Rose, Dell and Love 1987	21-51	Hooded Lister	M	EC-IC

Table 2:15 Chronological listing of all studies using a simple operant conditioning paradigm.
(NB IHC=Individually Housed Condition)

REFERENCE	TREATMENT AGE	STRAIN	GENDER	COMPARISON
Ough, Beatty and Khalili 1972	21-70	Holtzman	M F	EC-IC
Morgan and Einon 1975	25-120	Hooded Lister	F	SC-IC
Curry and Rose 1981	21-51	Hooded Lister	M	EC-IC
Rose and Lamden 1983	21-51	Hooded Lister	M	EC-IC
Rose, Dell, Love and Davey 1988	100-142	Hooded Lister	M	EC-IC-SC

Table 2:16 Chronological listing of all studies using a complex operant conditioning paradigm.

1985; Rose, Love and Dell 1986; Rose, Dell and Love 1987), whilst four studies have reported no significant differences between the groups (Gibson, Gill and Porter 1968; Ough, Beatty and Khalili 1972; Davenport 1976; Gluck and Pearce 1977). In only two of the studies was there an EC superiority. Will et al (1977) found EC animals bar pressed more, this difference only appearing, however, after several conditioning sessions and when the variable interval ratio was increased from VI15 to VI60. Freedman and Villeneuve (1981) employing brain stimulation as reward found response rates were increased in EC animals following injections of amphetamine or scopolamine.

The finding of increased responding in IC animals was unexpected and was initially attributed to increases in activity levels resulting from their early experience (Coburn and Tarte 1976). IC animals, it was postulated, were more active and therefore more likely to produce high levels of responding. More recently, however, Lamden and Rose (1979) have suggested that as a consequence of early deprivation the behaviour of IC rats is directed towards increasing contingent sensory input. The Skinner box paradigm, they suggest, provides a situation where correct responding maximises sensory stimulation and consequently produces higher levels of responding in the IC animal when compared with its EC counterpart. Indeed, in their experiment, where the reinforcer was gradually built up from a simple click from the lever microswitch, to include light and food, IC animals bar pressed significantly more than the EC animals, in all phases of the procedure.

Factors other than the sensory stimulation afforded an IC animal by bar pressing or differences in activity levels, IC animals being more active and thus more likely to bar press, might also explain the IC rates of responding. Morgan (1973) has suggested that isolates are more highly motivated for food reinforcement than socially housed animals because of their higher body weights (Fiala, Snow and Greenough 1977). This would be particularly important when animals were on a deprivation schedule and would predict that IC animals would bar press more to obtain more food. However, several sources of evidence do not support this hypothesis.

Firstly, bar press responding has been used by Coburn and Tarte (1976) and Morgan, Einon and Nicholas (1975) to compare the amount of lever pressing that persisted in the presence of free food. Coburn and Tarte (1976) have found IC animals acquire bar pressing behaviour more quickly and also demonstrate a greater preference for obtaining food via barpressing when compared with EC animals. However, IC animals consumed the same number of pellets as their EC counterparts suggesting that the increased lever pressing of the IC animals was not correlated with increased food intake. Consistent with these findings, Morgan, Einon and Nicholas (1975) investigated whether isolated and socially housed animals preferred lever-dependant or lever-independant food delivery. Free food was found to produce less lever pressing in socially housed animals, although when given the choice, isolates did not demonstrate a clear preference for either method. They did, however, leave more of the food pellets uneaten when they had to lever press for food. These findings argue against the interpretation that IC animals are simply more highly motivated for food reward. Indeed, as all the animals in this study were on ad libitum diets there should not have been any differences between the two groups in terms of food motivation, in this case. In a further experiment in the same study, the effect of food deprivation was to increase the relative amount of lever pressing in both groups, suggesting that the effects of increased food deprivation on lever pressing in the presence of free food were qualitatively different from the effects of social isolation. Morgan et al conclude therefore that the social/isolate difference cannot be interpreted as motivational.

Secondly, the findings of Lamden and Rose (1979) and Rose and Lamden (1983) also contradict the hypothesis that IC animals are more motivated for food reinforcement. Both these investigations employed a baseline testing period when lever pressing was unreinforced by either food or light. Under such conditions IC animals continued to bar press at a significantly higher rate than the EC group, suggesting that IC animals were less interested in the food reinforcement than in the intrinsic properties of the lever pressing per se.

Indeed, the effect of composite reinforcement on EC and IC animals has been extensively re-

searched by Rose, Love and Dell (1986) and Rose, Dell and Love (1987). The point of departure for their studies was the earlier finding of Lamden and Rose (1983) that one second of dim light plus food was more reinforcing for IC than EC rats, whereas one second of bright light plus food had equivalent reinforcement value for the two groups. Working with food deprived animals, Rose, Love and Dell (1986) found that using different light levels (low, medium or high) accompanied by the sound of the pellet dispenser but no food, produced higher levels of bar pressing in the IC groups when compared with their EC counterparts. Interestingly, the largest EC/IC difference emerged in the high light condition and was due to light reducing EC bar pressing rather than positively reinforcing IC bar pressing. When the composite reinforcer (sound of the pellet dispenser and light) was supplemented by the addition of food, bar press rates in both groups increased, but the higher light levels were still found to be negatively reinforcing for the EC groups. These findings provide clear evidence for an EC/IC difference in response to the composite reinforcers, IC animals being less influenced by variations in the intensity of response contingent light than their EC counterparts. These findings also suggest that the IC superiority observed by Lamden and Rose (1983) at one particular level of reinforcing light was due to a negative reinforcement effect of contingent light for EC subjects rather than to a positive reinforcement effect for IC subjects. One explanation for these differential reinforcement effects is that the EC and IC rats differ in their use of the response-contingent light period. For EC animals the light may simply be a neutral or even aversive response contingent event, whereas for IC animals it may have particular importance in providing an opportunity for visual exploration of the environment. Such a suggestion is consistent with the stimulus-seeking hypothesis (Lamden and Rose 1979; Chadha and Rose 1981) regarding EC/IC behavioural differences, namely that as a result of early partial sensory deprivation, the post-environment behaviour of IC rats is to some extent aimed at maximising sensory stimulation. In their second study, this hypothesis was tested directly by requiring subjects to bar press for darkness instead of light, thus removing the need to bar press to gain opportunities for visual exploration. As predicted by the stimulus-seeking hypothesis, EC/IC performance differences were eliminated (Rose, Dell and Love 1987).

Some studies, as mentioned earlier, have reported no significant differences in EC-IC lever press acquisition. Ough et al (1972), who were measuring CRF lever press acquisition incidentally to the initiation of a DRL20 schedule, recorded trials to a criterion of 50 reinforcements in a single session, and not actual lever press rates. As the majority of the studies reported earlier showed high levels of responding, that is more than 50 lever presses in *both* EC and IC animals, it is not surprising that no significant differences were found between the groups with respect to number of sessions required to achieve pretraining criterion levels. Considering next the studies by Davenport (1976) and Gluck and Pearce (1977), although neither study reported any acquisition rate differences, analysis of the extinction scores indicated that IC animals continued to bar press for no reinforcement longer than the EC groups. This finding, according to Joseph and Gallagher (1980), of a significant deficit in the ability of restricted rats to suppress an overlearned and formerly rewarded pattern of behaviour, might be due to the tendency in the IC animal to develop perseverative response hierarchies. Indeed, this explanation may well explain the findings of Gibson et al (1968) who reported that the recovery of pre-CER [12] response rates was faster in EC than IC animals, slower IC recovery reflecting their perseverative nature.

b) Complex Operant Procedures

To date there have been five studies that have compared differentially reared animals in complex operant learning situations (see Table 2:16). These studies have been concerned with either DRL or GO-NO-GO learning. In the former category, the differential reinforcement of low rates of responding or DRL schedule, reinforcement is contingent upon the occurrence of a response separated from the preceding response by at least some specified interval; on a DRL10 schedule, for example, only responses preceded by a 10 second pause are reinforced (Mackintosh 1974). Three studies have examined the effects of differential environments on DRL learning (Ough, Beatty and Khalili 1968; Morgan and Einon 1975: Curry and Rose 1981) and all have found that rats reared in isolation are deficient in the elimination of maladaptive responses, as required

[12] Conditioned Emotional Response

by the DRL schedule, when compared to animals raised in an enriched and socially stimulating environment.

Ough et al (1968) gave their animals 30 daily sessions on a DRL20 schedule and found that rats raised in an enriched environment exhibited a more efficient performance than the isolated animals. This difference in efficiency between the groups arose primarily because of the increased response rates of the isolates, who also earned fewer reinforcements. Interestingly, Ough et al suggest that the effects of social isolation cannot be accounted for in terms of changes in motivation produced by the differential rearing, as EC and IC animals were similar in body weight and responded at comparable rates for reinforcement on a CRF schedule. Instead they postulate that early social isolation produces a primary deficit in response inhibition (cf Morgan 1973), producing the patterns of responding in the IC animal which lowers their effeciency.

Morgan and Einon (1975) also found that isolates made more lever presses than social controls and obtained fewer rewards. Their experimental procedure, however, differed from that of Ough et al (1968), in that their animals were required to learn an alternating two lever DRL30 schedule, in which anticipatory [13] and perseverative [14] errors were recorded separately. This technique was employed to establish whether isolates were more perseverative than socially reared rats, the reasoning being as follows; if isolates have a tendency to repeat previously rewarded behaviour, an increase in perseverative errors would be predicted. If however,isolates were more food deprived, increases in both anticipatory and perseverative errors would ensue. Results supported the latter hypothesis, as social animals made more perseverative errors, whereas isolates made the two kinds of errors in equal numbers. Indeed, Morgan and Einon (1975) suggest that isolates are demonstrating a selective enhancement of responding in association with the expectation of reward and as a consequence are deficient in inhibiting responses when a low rate of responding is required.

In the final study to be described in this section, Curry and Rose (1981) investigated the nature of

[13] A press on the correct lever before the 30 second inter-reward interval
[14] A press on the lever that had last given a reward

reinforcement, and its effect on DRL learning. In their first experiment, reinforcement consisted of a pellet of food alone, and despite varying the motivational levels of the animals (deprivation versus satiation schedules) and the information given (a signalled time out period) no significant differences were found between the EC and IC groups. However, by making the reinforcement more complex, with the addition of a second of light, superior EC performance emerged as training proceeded. In this second experiment, no significant differences in mean total responses emerged between the groups, suggesting that the observed difference in performance was the result of a difference in efficiency with respect to the temporal distribution of responses.

The second type of complex operant learning to be reviewed is GO-NO-GO learning [15]. This paradigm typically involves training an animal on a fixed ratio schedule, whereby a subject must complete a fixed number of responses in order to obtain a reinforcement and then introducing a simple discrimination procedure, such that presence of a cue signals a "GO" period where responses are reinforced and absence of the cue signals a "NO-GO" period where responses are not reinforced. To date only two studies have examined the effects of differential environments on this paradigm, and both have found *no* significant differences in the performances of EC and IC animals in this task (Rose and Lamden 1983; Rose, Dell, Love and Davey 1988).

Rose and Lamden (1983) trained their animals on a two minute on/two minute off GO-NO-GO discrimination schedule where responses during the GO period, signalled by a two minute constant white noise, were reinforced by a composite reward (food and one second of illumination) according to an FR6 schedule. The light component of the reinforcer was "dim" for half the animals and "bright" for the other half. Neither the type of reinforcer, nor the environmental background of the animal affected GO-NO-GO performance. However significant differences emerged between the groups with respect to the number of bar presses. As with the simple operant procedures outlined above, IC animals bar pressed more than their EC littermates.

Probably the most complex paradigm to be employed in this literature is that of Rose, Dell, Love

[15] Although GO-NO-GO learning is a form of reversal learning, it is also a complex form of Skinner box learning and as such is reviewed in detail in this section

and Davey (1988), who used a GO-NO-GO *Reversal* discrimination problem with animals which had undergone differential post-operative environmental experience. The main thrust of their work was to separate out the processes of recovery and compensation following brain damage, by using a test in which compensation alone could not significantly reduce post-operative deficits. To achieve this, a unisensory task (light signalled GO-NO-GO reversal learning) coupled with unilateral lesions were employed. For the purpose of this present review, however, only the effects of the differential post-operative housing on the *sham-operated* animals are particularly relevant and as with the previous study (Rose and Lamden 1983) no significant differences were observed between the evironmental groups.

c) Summary of Operant Conditioning Findings

Considering first the acquisition phase of simple operant conditioning procedures, IC animals typically bar press more than their EC counterparts. This finding can be attributed to either higher activity levels in the IC animals (Coburn and Tarte 1976), or that their behaviour is directed towards maximising sensory stimulation (Lamden and Rose 1979; Rose, Dell and Love 1987). The notion that isolates are more highly motivated for food (Morgan 1973), however, has not been supported (Morgan, Einon and Nicholas 1975; Coburn and Tarte 1976; Lamden and Rose 1979; Rose and Lamden 1983). With respect to the slower extinction rates of IC animals, Joseph and Gallagher (1980) have argued that IC animals are displaying "a limited behavioural repertoire, characterised by a generalized tendency to overrespond, a propensity towards perseverating in repetitious patterns of limited and circumscribed responding, and a failure to habituate to repeated contact with novel stimuli" (p527), all of which would produce an animal less likely to extinguish a response as fast as its EC counterpart.

With respect to the more complex forms of operant behaviour, the picture is less clear and more task specific. Generally DRL experiments have found IC animals to be hampered by their earlier experiences, although whether this is due to a motivational deficit (Morgan and Einon 1975)

or to response inhibition (Ough et al 1968) is not obvious. GO-NO-GO learning, on the other hand, does not differentiate between the groups, suggesting that in some learning tasks, at least within the limits tested, early experience has neither a beneficial nor a detrimental effect on performance.

2:3 SECTION B: UNLEARNED BEHAVIOUR

In this section, the effects of exposing animals to differential environments will be examined with respect to the following behaviours:

1. GENERAL ACTIVITY LEVELS
    - Basal Activity
    - Reactivity
2. PERCEPTUAL ABILITIES
    - Depth Perception
    - Perception of Noxious Stimuli
3. MOTOR SKILLS
4. PLAY AND SOCIAL BEHAVIOUR
5. FEEDING AND SLEEPING BEHAVIOURS

2:3:1 GENERAL ACTIVITY LEVELS

The effects of exposing animals to either EC, IC or SC on ensuing general activity levels has been measured in a wide range of test situations including open fields, mazes, activity wheels, home cages and special enclosures (Munn 1950; Barnett 1975). This diversity in methodology reflects a considerable lack of consensus within the literature, regarding the precise definition of both the term "General Activity" and its underlying causes. In order to clarify this situation, Bindra (1961) has distinguished between "the spontaneous components of general activity" and "components which seem to be related to specific goals". Gross (1968) has also considered it necessary to distinguish between "basal locomotor activity", which consists of activity measured under constant and familiar environmental conditions and "locomotor activity" which he suggests comprises activity occuring after some environmental change. On the basis of such definitions, Lamden (1985) has postulated that general activity may be considered to have two distinct components, *basal activity* and *reactivity* and it is this distinction that will be employed in the present review.

a) Basal Activity

In order to examine basal activity, motor reactivity and goal directed behaviour must be eliminated from the test situation. To date only three studies (Baenninger 1967; Cummins et al 1978; Lamden 1985) have fulfilled this criterion, by examining home cage activity. These studies will be described in some detail below.

Considering Baenninger's (1967) study first, this was an observational study of the various developmental activities of group housed (SC) and isolated animals, from three to 92 days of age. Isolated animals were reported to engage in more exploratory locomotion, consummatory behaviour, attentive immobility, pawing behaviour and tail manipulation, whilst engaging in less sleep and rest than socially housed subjects. These group differences were consistent over time, with percentages of time subjects engaged in each category of behaviour varying with development. From these results it appears that significant basal activity differences are related to developmental experiences. However, when Lamden's (1985) work is considered, the picture is less clear cut.

Lamden's study differed in several respects from Baenninger's, in that she employed individually housed animals that had been exposed to either EC or IC for 30 days immediately prior to the experiment. Moreover, she used an automatic monitoring device, the "Actimat" (Marsden and King 1979) which has the facility to distinguish between high and low speed movements, as well as movements of part of the body as opposed to the whole body (Rose, Dell and Love 1985b). This apparatus removed any confounding observer effects, but in retrospect, was probably inappropriate, in that it could not categorise specific behaviours, unlike the earlier study.

Results from Lamden's work revealed no significant differences in activity levels between EC and IC animals, despite a tendency for IC animals to maintain higher levels of activity in the home cage when compared with their EC counterparts. This lack of statistical significance encompassed both total activity measures and time of day measures (that is activity during the

day as compared with night-time movements). No differences between the groups' night-time activity is particularily surprising considering that both Baenninger (1967), and later Tagney (1973) have reported that isolates sleep less than socially housed animals, and would therefore be active longer during night-time sessions. One solution to this apparent dilemma has been offered by Lamden herself, namely the sensitivity of the Actimat was such that it only recorded specific categories of gross movements and did not distinguish the more subtle differences that an observational study might identify.

The final study exploring baseline activity in the home cage (Cummins et al 1978) considered the effects of EC or SC on activity of groups of mice over a 23 day period. Quackenbush albino mice were housed in activity cages (N=6 per group) subdivided into three equal compartments. Food and water were available in the central compartment, passage between the compartments being recorded as the measure of activity [1]. For the enriched condition the two outer compartments were filled with a variety of toys, the social condition comprising the unadorned cages. Animals were injected with either saline, strychnine or chlorpromazine during the 23 day testing period, and overall, the social group was found to be less active than the enriched. This procedure is unusual in that it records group activity under drugged conditions rather than individual animals' activity as with Lamden's study. As a consequence, it is probably fair to say that to date no experiment has adequately explored the baseline activity levels of EC and IC animals in their home-cage. However, when the research on reactivity is taken into account, of these three studes it is Baenninger's findings which appear to be the most accurate.

b) Reactivity

In this section the components of general activity that display motor reactivity and goal directed behaviour in addition to basal activity will be examined in relation to the EC-IC-SC literature. As Barnett (1975) points out, however, in studying the many factors that influence activity, it

[1] Any mouse which crossed from one compartment to another triggered a photocell beam linked to an accumulative recording device.

becomes apparent that "different devices measure different phenomena". Consequently "we shall find ourselves obliged, if we wish to achieve rigour, to discard the unqualified term "activity" and to analyse the movements of animals into components precisely defined in terms of the procedures used and the observations made" (p31).

With this cautionary viewpoint in mind, the following review will outline the findings from the different *procedures* employed in the literature, whilst examining the different aspects of activity that each procedure has been attributed to be measuring. Consequently, this section is further subdivided into the following areas:

1. Open Field Procedures
2. Maze Procedures
3. Activity Wheels and Other Special Enclosures
4. Tests of Response to Novelty
5. Emergence Procedures

1: Open Field Procedures

Initially designed as a means of measuring emotional behaviour in the rat (Hall 1934; 1936) the open field test has since become one of the most widely used instruments in studying animal behaviour. Simplicity, ease of quantification and wide applicability are probably the prime determinants of its popularity (Walsh and Cummins 1976). Despite its status, however, the open field has survived for over 50 years with only two major reviews (Archer 1973; Walsh and Cummins 1976), although both the reliability (Ivinskis 1968) and validity (Ivinskis 1970; Royce 1977; Walsh and Cummins 1978) of its measures have been examined in some detail.

In essence, the open field test consists of the measurement of behaviours elicited by placing the subject in a novel open space, from which escape is prevented by a surrounding wall. The elicitation of these behaviours is dependant upon the interaction of the animal with a variety of test factors, outlined by Walsh and Cummins (1976), including "a) stimulation as a result of

removal from a familiar home environment, b) stimulation involved in transferring the animal to the open field, c) exposure to the test environment consisting of both the open field itself, and its surroundings and d) all prior experience of the test situation " (p482-483). In effect this last factor means that one is measuring, amongst other things, habituation and learning in response to the test environment. The magnitude of any particular behaviour elicited will therefore be a function of the multiway interaction of these factors. As yet their relative importance is almost completely unknown (Walsh and Cummins 1976) but there is evidence to suggest that each factor exerts a differential effect on animals of varying genetic and experiential backgrounds. It should be pointed out that any behavioural experiment measures responses to the above factors. However, in many studies, such as those reported earlier in this chapter, it is hoped that the subject will habituate to and hence be minimally influenced by, aspects of the test situation other than the specific stimulus component being used as *the* independant variable. In the open field on the other hand, the whole test situation, rather than any specific stimulus component is the independant variable and by its very nature must be multifactorial.

Unfortunately, variations in open field testing, with apparatus, techniques, subjects, analyses and interpretations diversifying enormously, have led to a disturbing lack of conformity in results in the open field literature as a whole (Walsh and Cummins 1976). This is particularly evident in the EC-SC-IC literature, where the difficulty of standardisation is futher compounded by the paucity of reports citing more than a small proportion of relevant procedural detail. Indeed, as can be seen from Tables 2:17, 2:18 and 2:19 which summarise the main findings of EC-IC, EC-SC and SC-IC comparisons respectively, there are no real consistencies in the results. For example, of the 19 EC-IC comparisons, ten have reported IC animals to ambulate more than their EC counterparts (Woods et al 1960; Levitsky and Barnes 1972; Fessler and Beatty 1976, Domjan et al 1977; Lamden 1985 (two studies); Dell and Rose 1987 (two studies); and Curry (1987), three have found no significant differences between the groups (Ray and Hochhauser 1969; Freeman and Ray 1972; and Joseph and Gallagher 1980) and six have reported an EC superiority in ambulation (Gill, Reid and Porter 1966; Gardner et al 1977; Studelska and Kemble 1979; Crnic

AUTHOR	STRAIN AND SEX	TEST AGE	PROC DETAIL	AMBULAT	REARS	BOLI
Woods, Ruckelshaus and Bowling 1960	Sprague Dawley M F	175	4 Days 2x5 mins	IC(F)>all		IC(M)>all
Gill, Reid and Porter 1966	Long Evans M		10 mins	EC>IC		
Ray and Hochhauser 1969	Zivic Miller M F		5 Days 4 mins	N/S	EC>IC	
Freeman and Ray 1972	Zivic Miller M F	88	5 Days 3 mins	N/S		IC>EC
Levitsky and Barnes 1972	M	49		IC>EC		
Gardner et al 1975	Long Evans M		3 mins	EC>IC		
Fessler and Beatty 1976	Holtzman M F	46-49	4 Days 3 mins	IC>EC	N/S	
Domjan et al 1977	Sprague Dawley		20 mins	IC>EC		
Studelska and Kemble 1979	Holtzman M		4 Days 3 mins	EC>IC	EC>IC	EC>IC
Joseph and Gallagher 1980	Zivic Miller M F	84	2x5 mins	N/S	N/S	N/S
Crnic 1983 1983	Sprague Dawley M	88-93	4 Days 3 mins	EC>IC	EC>IC	IC>EC
Rose et al 1985a	Hooded Lister M	58	5 Days 3 mins	IC>EC		
Lamden 1985	Hooded Lister M	58 58	5 Days 3 mins	IC>EC IC>EC	IC>EC	EC>IC EC>IC
Holson 1986	Long Evans M	101 (approx)	4 Days 3mins	SEC>IC		
Dell and Rose 1987	Hooded Lister F	58 133	5 Days 3 mins	IC> SEC IC>SEC	N/S IC>SEC	
Curry 1987	Hooded Lister M	58	5 Days 3 mins	IC>EC	N/S	EC>IC
Saari et al 1990b	Wistar	60	1 Day 3 mins	EC>IC	EC>IC	

Table 2:17 Chronological listing of all open field studies using an EC-IC comparison (PROC= Procedural, AMBULAT= Ambulation)

AUTHOR	STRAIN AND SEX	TEST AGE	PROC DETAIL	AMBULAT	REARS	BOLI
Dawson and Hoffman 1958	Wistar M F	30 50	4 mins	EC>SC N/S		N/S N/S
Denenberg and Morton 1962	Wistar M F	180	6 Days 3 mins	N/S		SC>EC
Duke and Seaman 1964	Albino M F	100 200	5 mins	EC>SC		
Denenberg and Morton 1964	Purdue Wistar M F	70	3 mins	EC>SC		
Denenberg and Whimbey 1968	Purdue Wistar M F	220	4 days 3 mins	SC>EC		
Denenberg and Rosenberg 1968	Purdue Wistar F	200	4 Days 3 mins	N/S		N/S
Manosevitz 1970	Mice random bred	38	5x2 mins	EC>SC		SC>EC
Finger and Fox 1971	Rattus Norvegicus M	60	2x15 mins	SC>EC		
Manosevitz and Montemayor 1972	A/J, C3H/HeJ C57BL/10J	41	5 Days 2 mins	EC>SC		
Manosevitz and Joel 1973	Mice M F	41	5 days 2 mins	EC>SC		SC>EC
Mitani 1975	M F	104	3x5 mins	EC>SC		N/S
Huck and Price 1975	Norway M F		5x15 mins	EC>SC	EC>SC	SC>EC
Fessler and Beatty 1976	Holtzman M F	46-49	4 Days 3 mins	SC>EC	N/S	
Sjoden and Soderberg 1975	Wistar M F	210	4 Days 4 mins	EC>SC	EC>SC	N/S
Studelska and Kemble 1979	Holtzman M		4 Days 3 mins	EC>SC	N/S	N/S
Rose et al 1985a	Hooded Lister M	58	5 Days 3 mins	SC>EC		

Table 2:18 Chronological listing of all open field studies employing an EC-SC comparison.

AUTHOR	STRAIN AND SEX	TEST AGE	PROC DETAIL	AMBULAT	REARS	BOLI
Stern et al 1960	Sprague Dawley M	58 65	2 mins	SC>IC	SC>IC	SC>IC
Ader and Friedman 1964	Sprague Dawley M	85 105	2 min 1 min	SC>IC		SC>IC
Archer 1969	Wistar F Wistar M Wistar F	6 weeks 28 weeks 28 weeks	10 mins 10 mins 10 mins	N/S SC>IC N/S		
Syme 1973	Hooded F	10	10 mins	IC>SC		
Einon et al 1975	Hooded Lister M F	25 45	4x3 mins	N/S IC>SC		
Fessler and Beatty 1976	Holtzman M F	46-49		N/S	N/S	
Morgan and Einon 1976	Hooded Lister	45	7x3 mins	IC>SC		
Einon and Morgan 1978a	Hooded Lister M F	60	7x3 mins	IC>SC		
Einon et al 1978	Hooded Lister F	45	2x3 mins	SC>IC		
Studelska and Kemble 1979	Holtzman M		4 Days 3 mins	SC>IC	SC>IC	SC>IC
Benton and Brain 1981	Mice		3 Days 5 mins	SC>IC		SC>IC
Chivers and Einon 1982	Mustela Furo		2 Days 2x2 mins	IC> SC		
Rose et al 1985a	Hooded Lister M	58	5 Days 3 mins	N/S		

Table 2:19 Chronological listing of all open field studies using an SC-IC comparison.

1983; Holson 1986; Saari et al 1990b). This lack of consistency is augmented when the EC-SC and SC-IC comparisons are taken into account, with ten out of 17 studies reporting EC animals to ambulate more than their SC counterparts, five studies showing IC to be superior to SC animals and six studies the opposite, namely SC superiority over IC animals.

One approach to disintangling the diversity found in these studies has been to consider the *individual* effects of subject variables, physical parameters of the field and of the test environment, on open field performance. Unfortunately, however, the results of this type of analysis have not proved to be efficacious. In her review, Lamden (1985) meticulously examined EC-SC-IC open field results, taking into account age, sex and strain of animals, size, shape and colour of the field, levels of background noise and illumination and reported "that no firm conclusions can be drawn regarding the true influence of (these) factors... on EC-IC differences in open field behaviour" (p186).

A complete understanding of the effects of differential environments on open field performance is further complicated by the fact that the behavioural measures recorded, such as ambulation, rearing and defecation to name but three, have been differentially interpreted as evidence of a variety of underlying constructs including emotionality, arousal, fear and exploration, or on the basis of the behaviour's presumed "purposive or adaptive nature" (Walsh and Cummins 1976 p500). Underlying constructs may be suggested by the face validity or anthropomorphic interpretation of a particular behaviour, by resemblance to a natural behaviour pattern or to other constructs, or by factor analysis. The relationship between these underlying constructs and the dependant variables typically measured in the open field, has, however become increasingly more difficult to interpret. For example, activity was originally found to be negatively correlated with the construct "emotionality" (Hall 1936) and the dependant variable defecation. However, open field activity is now known to be factorially complex, with significant loadings on both an emotional reactivity factor and an exploratory factor (Whimbey and Denenberg 1967b). In particular, high activity scores on the first day of open field testing are now thought to indicate

high emotional reactivity, whereas high activity levels from day two onwards reflect low emotionality. Thus high activity on day one can mean high emotionality (Salama and Hunt 1964) high exploratory behaviour (Hayes 1960) or both (Whimbey and Denenberg 1967b)

This factorial complexity makes for difficulty in interpreting activity findings, especially when subjects are tested for only one day in the open field. Indeed, several researchers (Stern et al 1960; Duke and Seaman 1964; Syme 1973; Manosevitz and Joel 1973) have given their subjects one open field trial and interpreted high activity as indicative of emotional reactivity. Such an interpretation is questionable, when activity is the only data considered. Whimbey and Denenberg (1967b) recommend that subjects be tested for a number of days and that both activity and defecation be taken into account, when attempting to give some conceptual meaning to open field performances.

Over the last couple of decades, more information pertaining to the analysis of open field data *over trials* has emerged in the literature and from these studies a clearer picture of the effects of differential environments on open field performance has become available. For example, when activity scores are observed over days, it becomes apparent that the IC animal's behavioural profile is different from either its EC (Freeman and Ray 1972; Joseph and Gallagher 1980; Rose, Dell and Love 1985a; Dell and Rose 1987) or SC (Morgan and Einon 1976; 1978; Einon, Morgan and Kibbler 1978) counterparts. Typically, over trials, the IC animal maintains a high level of activity, whereas EC and SC animals' activity levels drop (Einon, Morgan and Sahakian 1975; Morgan and Einon 1976; Einon, Morgan and Kibbler 1978; Joseph and Gallagher 1980; Lamden 1985), which may well reflect their failure to inhibit established behaviour patterns. This effect has also been observed within a trial (Domjan et al 1977), where over 20 minutes, deprived subjects were found to cross more squares and remain more active during the latter part of the test session, than subjects which had been reared in the enriched environment. This behaviour pattern has been attributed to both environmental enrichment reducing the novelty of various stimuli, thus reducing exploratory behaviour in the open field (Domjan et al

1977) and to the fact the impoverishment might result in an abnormal developmental process (Einon, Morgan and Sahakian 1975), preventing appropriate responding. The latter idea has been developed further (Morgan and Einon 1976; Benton and Brain 1981), with high levels of activity in isolates being attributed to a general disturbance of their inhibitory mechanisms, and/or hyperarousability (Einon and Morgan 1978a). Interestingly, Morgan and Einon (1976) when reporting the extraordinarily high levels of activity in their isolates, mention that this activity was qualitatively different from their social controls and suggest that it could *not* be considered exploratory in nature [2].

Another widely used measure of activity is "rearing", which has proved a reliable (Ivinskis 1968) and valuable measure. Combined with ambulation it has proved to reflect a stable individual trait, "non-specific excitability level" (Walsh and Cummins 1976), and has been found to correlate highly with ambulation (Ray and Hochhauser 1969). As with ambulation, it has also been employed as a measure of exploration (Dell and Rose 1986), and suffers from the same problems of interpretation. Within the EC-SC-IC literature, rearing is less commonly measured than ambulation, however, the same lack of consistent results emerge. As can be seen from Tables 2:17, 2:18 and 2:19, EC animals rear more than IC animals in four out of ten studies (Ray and Hochhauser 1969; Studelska and Kemble 1979; Crnic 1983; Saari et al 1990b) but less than IC animals in two out of ten studies (Lamden 1985; Dell and Rose 1987), the remaining studies reporting no significant differences between the groups. With respect to the EC-SC comparisons, of the four studies reporting this measure, two have found EC animals to rear more (Huck and Price 1975; Sojden and Soderberg 1975) than their SC counterparts and two report no differences between the groups (Fessler and Beatty 1976, Studelska and Kemble 1979). SC-IC comparisons further complicate the picture with two out of three studies reporting an SC superiority (Stern et al 1960; Studelska and Kemble 1979) and one out of three, no significant differences between the groups (Fessler and Beatty 1976). As with ambulation, probable causes of these diverse results lie in the variations of test environment, physical characteristics of the apparatus and subject

[2] An alternative explanation has been advanced by Rose et al (1986) who suggest that the IC animals may be stimulus seeking.

variables. These factors will also underly the lack of consistency in defecation scores, the last behavioural measure to be considered in this section.

Number of fecal boli, as previously mentioned, is one of the dependant variables that has been factorially associated with emotionality (Whimbey and Denenberg 1967b), although it has been anthropomorphically associated with this construct from the inception of the open field as a behavioural test (Hall 1934). The validity of defecation as an index of emotionality has also been examined as has its reliability (Ivinskis 1968), although the adequacy of the criteria used in this study has been questioned (Walsh and Cummins 1976). As can be seen from Tables 2:18 and 2:19, SC animals defecate more than IC animals in four out of four studies where this measure was taken and defecate more than EC animals in four out of ten studies, with with the remaining six studies reporting no significant differences between the groups. This suggests that SC animals are more emotional than either their EC or IC counterparts. With respect to the EC-IC comparisons, however, with EC animals defecating more than their IC counterparts in four out of eight studies (Studelska and Kemble 1979; Lamden 1985; Dell and Rose 1987; Curry 1987) and IC more than EC in three out of eight studies (Woods et al 1960; Freeman and Ray 1972 Crnic 1983) the picture is less clear.

In this section, the effects of differential experience on open field performance have been outlined and information presented in terms of both the dependant variables measured and the postulated underlying constructs. These constructs and variables are not unique to the open field procedure, however, and will be dealt with again in the following section, which considers activity, emotionality and exploration measured in mazes and related procedures rather than in the open field.

2: Mazes and Related Procedures

As can be seen from Table 2:20, the effects of differential environments on reactivity and exploration, have been examined in a variety of maze procedures [3], including *elevated mazes* (Forgus 1954; Luchins and Forgus 1955) *Y mazes* (Luchins and Forgus 1955; Montgomery and Zimbardo 1957; Zimbardo and Montgomery 1957; Ehrlich 1959; Forgays and Read 1962; Inglis 1975) *Hebb-Williams mazes* (Woods et al 1960; Wells 1970; Chadha and Rose 1981; Dell and Rose 1986) *Dashiells maze* (Ehrlich 1959) and *Davenport mazes* (Joseph 1979; Joseph and Gallagher 1980). In addition, four studies (Hoffman 1959; Moyer and Korn 1965; Greenough et al 1972a; Chadha and Rose 1981) have used a maze-related procedure, namely runway training, which for convenience sake will also be included in this section. As with the open field studies detailed in the previous section, subject and procedural differences in the present literature have led to a diversity of results and explanations, which will be examined in some detail in the following pages.

Initial interest in the reactivity and exploration of animals in mazes stemmed from the work of Hebb (1947; 1949) which suggested that the organisation of adult behaviour was largely determined by the quality of infant experience and learning. Early experiments (Forgus 1954; Luchins and Forgus 1955) reported that subjects with richer experiences showed greater activity in terms of the number of units of a maze traversed and less fear or emotionality, in terms of number of defecations than a control group raised in a "meager" environment. However, a later experiment (Zimbardo and Montgomery 1957) found the opposite, namely that rats reared in normal laboratory cages explored a Y maze significantly more than rats reared in an environment offering complex sensory and proprioceptive stimulation. This apparent contradiction can, however, be attributed to the fact that Forgus and his associates employed "handling" as an additional factor in their enriched experience. This variable alone would account for control animals, who were not handled, being more frightened and emotional, which would result, according to Zimbardo

[3] In this section only those studies in which *activity* was measured will be included, for learning performance the reader is referred to section 2:2.1.

AUTHOR	COMPARISON	STRAIN SEX	TEST AGE	TEST ENVIRON	TEST PROCED	ACTIVITY EXPLORATION EMOTIONALITY
Forgus 1954	CVP/CV-SC	Hooded Lister M	92	Elevated Maze	1x7 mins	Units Entered CVP=CV>SC Defecation SC>CVP/CV
Luchins and Forgus 1955	EC-SC EC-SC	Hooded Lister F	60 60	Elevated Maze Y Maze	1x5 mins 2X5 trials	Units Traversed EC>SC Defecation SC>EC Variability EC>SC
Montgomery and Zimbardo 1957	NC-BD-SBD	Wistar M F	26 51 101	Y Maze	4x10 mins 4x10 mins 4x10 mins	Units Traversed all N/S Defecation all N/S
Zimbardo and Montgomery 1957	NC-FE	Wistar M F	26 51 101	Y Maze	4x10 mins 4x10 mins 4x10 mins	Units Entered NC(F)>others FE(M)>NC(M)
Hoffman 1959	FE-SC-IC	Wistar M F	70	Runway	3x4 mins	Lines Crossed N/S Defecation N/S
Ehrlich 1959	FE-SC	Hooded M	76	Y Maze Dashiells	3x10 mins 3x10 mins	Units Entered SC>FE
Woods et al 1960	FE-SC	Sprague Dawley M F	175	Hebb Williams	4x10 mins	Units Entered SC(F)>rest Defecation SC(M)>rest
Forgays and Read 1962	FE-SC	Albino M	114	Y Maze	2x5 mins	Units Entered N/S Defecation N/S
Moyer and Korn 1965	SC-IC	Albino M	111	Runway	4 mins	Activity N/S Defecation SC>IC
Ravizza and Herschberger 1966	SC/C-SC/NC	Albino M F		Table Top	5x5 mins	Activity SC/NC>SC/C Defecation SC/NC>SC/C
Wells 1971	EC-IC			Hebb Williams		Units Entered IC>EC
Greenough et al 1972a	EC-HC-IC	Long Evans M	54	Runway	8 trials	Speed EC/HC>IC
Inglis 1975	EC-SC	Hooded Lister M F	126	Y Maze	15 mins	Sitting EC(M)>rest Grooming IC(M)>rest Defecation N/S
Joseph 1979	EC-IC	Holtzman M F		Davenport Maze	4x12 trials	Maze Entries EC>IC
Joseph and Gallagher 1980	EC-IC EC-IC	Zivic Miller M F	84 86	Closed Field Davenport Maze	10 mins	Units Entered IC>EC Units Entered EC>IC
Chadha and Rose 1981	EC-IC	Hooded Lister M F	64 68	Runway Hebb Williams	4 trials 1 trial	Speed IC>EC Zones Entered IC>EC
Warren et al 1982	EC-IC	C57BL/6J M	750	Brightness Discrimination		Units Crossed N/S Latency N/S
Dell and Rose 1986	EC-IC	Hooded Lister M	80	Hebb Williams	6x8 trials	Units Entered IC>EC Rears N/S

TABLE 2:20 describes those studies using maze based procedures, to measure activity, exploration and emotionality. (CVP=complex visual and proprioceptive, CV=complex visual, MVP=minimal visual and proprioceptive, NC=normal cage, BD=behaviourally deprived, SBD=sensorily and behaviourally deprived, FE=free environment, SC/C=social with climbing facilities, SC/NC=social with no climbing facilities, HC=handled)

and Montgomery (1957) in an inhibition of activity and exploration in an elevated maze. This was confirmed by Ehrlich (1959), who found handling significantly increased exploration in her subjects.

The finding that normal cage animals explored more than their free environment counterparts, was explained in terms of the novelty of the test environment for the two groups of rats. In particular, for animals reared in large cages offering a wide range of perceptual, locomotor and social experiences, the simple Y maze may be less novel than it is for rats reared in laboratory cages and therefore evoke less exploratory behaviour (Zimbardo and Montgomery 1957). For free environment subjects, it may be necessary to have a more complex test environment, one at their acquired level of experienced stimulation, in order to evoke a high rate of exploration. Indeed, these results are predicted by the Dember-Earl (1957) theory of behaviour, which states that the past experience of a subject affects the "complexity" of that subject and therefore the range of stimuli to which he/she will respond. High levels of environmental stimulation will result in a more complex subject, who will respond with less frequency or amplitude to stimulus situations that are less complex than their normal environment.

Of particular interest, however, was the speculation that emerged from this research, that the apparent superiority of free environment animals in Hebb-Williams maze performance, may well reflect a reduced level of exploration in these subjects, because of the decreased novelty of the test situation. This reduction in exploration would result in less errors as the animals traversed the maze and suggested that environmental rearing was producing differences in exploratory drive, rather than in intelligence. Woods (1959) presented evidence which supported this line of enquiry, when he reported that restricted animals were more likely to retrace through the maze from the goal box before returning to eat and thus terminate the trial. In a further experiment (Woods et al 1960) exploratory behaviour was measured in the Hebb-Williams apparatus, and was found to correlate significantly with Hebb-Williams error scores, leading the researchers to conclude that "exploratory differences and not intelligence differences seem to be a major factor in the

characteristic finding that Ss reared in a free environment are superior problem solvers when compared with Ss reared under restricted conditions" (p 199). Interestingly, they also found that after adaption and preliminary training in the Hebb-Williams maze, restricted groups explored *more* than they did initially, and the free group explored *less*. This maintenance of high levels of exploratory behaviour in restricted animals is reminiscent of the high levels of activity over trials of these animals in the open field literature (Joseph and Gallagher 1980; Lamden 1985).

The notion that restricted rats do poorly on maze learning tasks because of excessive exploration has been questioned (Joseph 1979; Joseph and Gallagher 1980). In both these studies enriched animals were found to explore more than their restricted counterparts. Joseph and Gallagher (1980) suggest that these differences reflect a perseverative tendency in restricted animals such that repetitive sequences of movement (for example a particular pathway across the maze) once learned, persist and compete with other behaviours. This IC failure to inhibit inappropriate responses has also been suggested by Dell and Rose (1986) as an explanation for their findings of EC/IC differences in post asymptotic performance, but not in initial learning in a Hebb-Williams maze. Additionally, in their experiment, reasoning that exploration as measured by squares entered would be inextricably linked with error scores, they included an additional measure of exploration, namely rearing and reported no significant differences between the groups.

As can be seen by the above, interest in activity and exploration in mazes has been a secondary issue to the more usual focus of interest, namely EC/IC differences in learning. However, some research has investigated the effects of differential environments on reactivity alone, whilst using a maze procedure (Inglis 1975). In this study, animals were exposed to differential environments when mature, and then tested for exploration in a Y maze. Interestingly, and in contradiction to earlier findings (Zimbardo and Montgomery 1957), higher levels of exploration as measured by activity scores and other behavioural parameters, were found in the enriched animals. However, it should be pointed out that previous experiments typically employed environmental stimulation in a developing organism and age of experience may well be the important factor in this type of

behaviour. Indeed, in the case of the rat it would seem to be biologically maladaptive for the naturally occuring levels of infantile stimulation to set the adult level of exploratory behaviour, as in the wild, the natural rearing habitat of the young may be considered to be impoverished (Daly 1973).

Finally, as can be seen from Table 2:20, emotionality as measured by defecation in the apparatus has also been investigated. Generally, if there are significant differences between the groups, enriched animals are reported as less emotional than their group-housed or restricted counterparts.

3: Activity Wheels and Related Enclosures

There have been some studies of activity and exploration that have used *automatic* recording devices and these constitute the basis of this present section. In particular, experimenters have employed activity wheels, photocell cages and in one instance (Lamden 1985), an activity monitor based on Doppler shift radar, developed by Marsden and King (1979). Each of these procedures will be described separately below.

The activity wheel was first used by Stewart in 1898 (cited in Reed 1947), and according to Miezejeski et al (1976) is the most frequently used measure of general or spontaneous activity in the animal literature. With respect to the EC-SC-IC literature, the first reported use of this apparatus was in a series of studies by Whimbey and Denenberg (1966; 1967a) investigating the effects of four experiential variables, namely a) type of mother, b) presence or absence of handling in infancy, c) type of rearing environment preweaning and d) postweaning environment, on 23 behavioural tests of which one was activity wheel behaviour. Mean scores of each of these 23 tests were intercorrelated and factor analysed and the results were interpreted as establishing the importance of manipulations early in life in generating stable and relatively permenant complex intergroup differences. Unfortunately, no individual test score was presented in this literature, so the effects of differential environments on activity wheels are inaccessible from this data. As can be seen from table 2:21, however, there are six studies that do detail the results of EC-SC-IC

AUTHOR	COMP	STRAIN SEX	TEST AGE	TEST ENVIRONMENT	TEST PROCEDURE	FINDINGS
Ravizza and Herschberger 1966	SC/C vs SC/NC	Albino M F	145	Wahmann Activity Wheel	24 hours	SC/C> SC/NC F>M
Manosevitz 1970	EC-SC	Mus Musculus M F		Wahmann Activity Wheel	4 days	EC>SC
Manosevitz and Montemayor 1972	EC-SC	A/J, C3H/HeJ C57BL/10J M F	60 66	Exploration Apparatus Wahmann Running Wheel	5 days 10 mins 30,60,90 mins 24 hours	SC>EC EC>SC
Levitsky and Barnes 1972	EC-IC	Rats M	119	Photocell OpenField	20 mins	IC>EC
Manosevitz and Joel 1973	EC-SC	Mus Musculus M F	95-100 85-90	Exploration Apparatus Wahmann Running Wheel	5x10 mins 30,60,90,120 mins 4 days	SC>EC EC>SC 1st 120 mins EC>SC
Manosevitz and Pryor 1975	EC-SC	C57BL/6J M F	41 50 53	Open Field Wahmann Activity Wheel Exploration Apparatus	5x2 mins 30,60,90,120 mins 24 hours 5x10 mins	Activity EC>SC Boli SC>EC F>M F>M
Sahakian et al 1975	EC-IC	Hooded Lister M F	adult	Photocell Cages	2 hours	IC>EC
Mitani 1975	EC-SC	Rats M F	104	Activity Wheel	20 mins 2 days	N/S
Sahakian et al 1977	SC-IC	Hooded Lister F	60-70	Berlyne Box	1 hour	IC>EC
Will et al 1977	EC-IC	August Fischer M F		Photocell Actographic	2 hours	F>M
Einon and Morgan 1978	SC-IC	Hooded Lister F	125	Photocell Cage	1 hour	IC>SC
Einon et al 1978	SC-IC	Hooded Lister F	45	Activity Cage	1 hour	IC>SC
Einon and Sahakian 1979	SC-IC	Sprague Dawley F	70	Photocell Cage	1 hour	IC>SC F>M
Joseph and Gallagher 1980	EC-IC	Zivic Miller M F	80	Running Wheel	4 days 15 mins	F>M
Gentsch et al 1981	SC-IC	Wistar M	56,70, 84,105	Activity Cage	84 hours	SC>IC
Sahakian et al 1982	SC-IC	Hooded Lister F	20,22,24: 42,45: 14,15,18,20 23,25,27	Photocell Cages	0.5 hour 24 hours 0.5 hour	IC>SC N/S IC>SC
Lamden 1985	EC-IC	Hooded Lister M	62	Square T Shape Rectangular	5x3 mins 5x3 mins 5x3 mins	IC>EC IC>SC IC>EC

TABLE 2:21 details those studies employing activity wheels and procedures employing activity monitors such as photocell beams. (COMP= Comparison)

on activity wheel performance and of these four have found EC animals to be significantly more active than their SC counterparts (Ravizza and Herschberger 1966; Manosevitz 1970; Manosevitz and Montemayor 1972; Manosevitz and Joel 1973). Two have reported no significant differences between the environmental groups, but have found female mice (Manosevitz and Pryor 1975) and female rats (Joseph and Gallagher 1980) to be significantly more active than their male counterparts. The finding that restricted animals are less active in the running wheel has been interpreted as evidence of Lore's (1968) habituation hypothesis, whereby sensory restriction "produces an animal which is more emotionally reactive and which habituates more slowly to novel environments" (p571). According to Manosevitz and Joel (1973), " if one assumes that rearing in the control cages provided some degree of sensory restriction relative to the experiences of the mice reared in the enriched environment, then the control mice... would be expected to habituate more slowly to the novelty of the running wheel... The animal that habituates slowly would spend more time in the home cage and less time running in the wheel. Therefore, slower habituation in the running wheel among the control animals would lead to lower activity scores" (p380).

Failure to habituate may also explain the results of the findings from the enclosures employing photocell/photobeam mechanisms, or radar based activity monitors. In these procedures, animals cannot "hide" in a home cage and are constantly monitored in the test environment. Consequently, any slow habituation to the environment may well result in higher activity scores. Indeed, this does appear to be the case. Of the twenty experiments reported in the literature, thirteen have found the most restricted group to be significantly more active in the apparatus, than their comparison group. In particular, two experiments (Manosevitz and Montemayor 1972; Manosevitz and Joel 1973) working with mice, found SC animals to be more active in an exploratory apparatus than their EC counterparts, six experiments (Levitsky and Barnes 1972; Sahakian et al 1975; 1977; Lamden 1985-three experiments) found IC rats to be more active than EC rats in photocell or radar monitored cages and five experiments (Einon and Morgan 1978; Einon et al 1978; Einon and Sahakian 1979; Sahakian et al 1982-two experiments) found IC

animals to be more active than their SC counterparts, again in photocell monitored cages. Only two studies (Manosevitz and Pryor 1975; Gentsch et al 1981) have found restricted animals to be less active than their more enriched counterparts, with the remaining experiments (Manosevitz and Pryor 1975; Mitani 1975; Will et al 1977; Joseph and Gallagher 1980; Sahakian et al 1982) reporting no significant differences between the groups.

A second explanation of these findings lies in the discrepancy hypothesis proposed by Kessen (1968). This hypothesis suggests "that animals raised in complex environments develop perceptual skills or strategies for processing information or (and the alternatives are not mutually exclusive) animals raised in restricted environments are unable to handle the discrepancy between the limited range of their early experience with the typical variety and complexity of test environments" (p396). In the experiments where the animals' behaviour is monitored directly, according to Manosevitz and Joel (1973), this hypothesis would predict that as the apparatus does not provide a high degree of novelty for the enriched animal, exploration will decline after the initial trial, whereas the more restricted animal will continue to explore the apparatus, despite the reduction in novelty over time. In fact, this reaction of the isolated animal to novelty does appear to be substantiated (Sahakian et al 1975; 1977; 1982) and will be dealt with in more detail in the following section, which examines the effects of differential experience on novel object contact and exploration/activity in novel environments.

In summary, therefore, monitoring of activity levels of EC-SC-IC animals has revealed higher levels of spontaneous activity in the EC animal, as measured in an activity wheel, but lower levels of EC reactive activity (Gentsch et al 1981), when placed in a novel enclosure. Whether this reflects genuine and differing activity baselines for the various rearing conditions, or an impoverished deficit in habituation, is still unresolved.

4: Tests of Response to Novelty

Exposing an animal to a novel environment produces behaviour which, according to Whimbey and Denenberg (1967b) "may be motivated by its exploratory tendencies, its emotional reactivity, or both" (p503) [4]. In this section the effects of differential environments on an animal's reaction to novelty will be reviewed. Obviously, removing an animal from its home cage and placing it in any test environment for the first time constitutes a novel experience [5]. In this section, however, only those studies that *specifically* examine the effects of novel environments and novel objects on an animal's behaviour will be included, although in some instances, references may be made to procedures outlined in earlier sections.

One of the earliest reports on the effects of past experience on the rats response to novelty, was that of Ehrlich (1961). Having found in a previous experiment (Ehrlich 1959) that there was a tendency for rats from a restricted environment to explore more in the Y and Dashiells mazes than rats raised in a free environment, she tested differentially housed animals' responses to novelty in a Skinner box. Restricted rats bar-pressed significantly more for stimulus change than did their enriched counterparts, which has also been found in later work (Rose, Dell and Love 1987). These results were interpreted as demonstrating the inadequacy of Myers and Miller's (1954) theory of exploration, which states that animals explore because of an internal "boredom drive" aroused by monotonous surroundings. According to Ehrlich (1961) Myers and Miller's theory fails to take into account the animal's past experience.

Since this early work, the effects of differential experience on an animals response to novelty have been examined in a variety of environments, including open fields (Syme 1973; Einon and Morgan 1976; Sahakian et al 1977; Einon and Morgan 1977; Einon et al 1978; Joseph and Gallagher 1980), specialised arenas (Woods and Davidson 1964; Lore and Lovowitz 1966; Konrad and Bagshaw 1970; Morgan 1973; Turpin 1977; Morgan et al 1977; Will et al 1979; Chivers and Einon 1982;

[4] As noted earlier in the section on Open Field procedures, these authors (Denenberg and Whimbey 1968) consider the constructs of emotionality and exploration to be factorially distinct.

[5] Indeed, exploration engendered by this novelty has been employed as an explanation for high error scores in impoverished animals' Hebb-Williams maze performance.

AUTHOR	STRAIN SEX	COMP AGE	TEST ENVIRON	TEST PROC	FINDINGS
Ehrlich 1961	Hooded M	EC-SC	Skinner Box	20 mins 6 days	No of bar presses: SC>EC
Woods and Davidson 1964	Sprague Dawley M	CE-SE adult	Rearing Cage	30 mins	Sniffing: Determinant of exploration is increased with complexity
Lore and Levowitz 1966	Wistar M	EC-IC 121	Object in cage	15 mins	Forced objectcontact: IC>EC
McCall et al 1969	Holtzman F	EC-IC 77	Modified Hebb Williams Objects and No Objects	12 Days 5mins	Time in field: EC>IC Time with Object: EC>IC (Only with caretaker present) EC=IC (No caretaker) Differentiation of novel Objects: EC>IC
		EC-IC 144		12 Days	Differences in Explor. style
Konrad and Bagshaw 1970	Cats F	EC-IC 15 months	Room with Novel Objects	12 Days 15 mins	Room Exploration: Initially EC>IC Object Contact: Initially EC>IC
Morgan 1973	Hooded Rats M F	EC-IC 80-110 EC-SC 80	Moving Ball	14 days Pulling 7 days Pushing	EC=IC EC>IC N/S: ECF>SCF
Syme 1973	Hooded F	SC-IC-C 190	Open Field Activity Platform	10 mins 5 mins	Squares entered: IC/C>SC Activity: C=IC>SC
Einon and Morgan 1976	Hooded Lister M F	SC-IC 60	Object Contact in Open Field	0-3 mins 5-7 mins 0-3 mins 13-16 mins 26-29 mins	Variety of Objects Contacted: SC>IC SC>IC IC>SC SC=IC
Sahakian et al 1977	Hooded F	SC-IC 60-70	Berlyne Box with Objects	2 days 10 mins	SC's contact with objects diminished over days No effect with IC's Bouts of exploration: IC>SC Activity: IC>SC
		SC-IC	Open Field half=novel	10 mins	Time spent in novel half of field: IC>SC Activity: N/S
Morgan et al 1977	Hooded Lister M F	SC-IC 55	Pulling Ball	5 Days 6 Trials	IC>SC
Turpin 1977	Long Evans M	SC-IC	2 Boxes Horizontal Vertical Stripes	30 mins	IC more time in familiar environ SC more time in novel environ
Einon and Morgan 1977	Hooded Lister M F	SC-IC 46	Open Field with objects	0-3 mins 4-7 mins 17-20 mins 30-33 mins	SC-IC between 15-25,25-45 days early environments N/S 25-45 increased IC object contact IC lower rate decline of contact
		SC-IC 91	as above	as above	SC-IC between 25-45,46-90 days 25-45 increased IC contacts
		SC-IC 181	as above	as above	SC-IC between 25-90, 91-180 days 25-90 increased IC contacts
File 1978a	Hooded Lister M	EC-SC-IC 73-78	Objects in Home cage	3x5mins	No of objects Contacted: N/S

Table 2:22 details those studies employing novel environments and novel objects.

AUTHOR	STRAIN SEX	COMP AGE	TEST ENVIRON	TEST PROC	FINDINGS
Klippel 1978	Mus Musculus	EC-SC-IC	Greek Cross	2 mins	Time spent in each box: EC>SC/IC No of box entries: EC>SC/IC
Einon et al 1978	Hooded Lister F	SC-PI-IC 50	Open Field with objects	0-3 mins 4-7 mins 17-20 mins 30-33 mins	PI behave like IC N/Sdiffs in no of object contacts, but differences in nature of contacts
Will et al 1979	August M	EC-SC 87	2 linked boxes one=objects		No of boli in boxes: SC preferred empty box Video of animals: EC/SC preferred empty box
Ferchmin et al 1980	Rattus Norvegicus M	EC-IC 89 59	Greek Cross	2x5mins	No of entries: EC>IC EC>IC
Joseph and Gallagher 1980	Zivic Miller M F	EC-IC 86	Open Field barriered		Section entries: EC>IC
Chivers and Einon 1982	Mustela Furo M F	SC-PI-IC 147	Room with Objects	2x1 hour	Object contacts: IC/PI>SC
Renner 1984	No Details	EC Juvenile Young Adults	Arena with Objects	No Detail	EC-IC animals employ diff strategies Bout diversity EC>IC
Renner and Rosenzweig 1986a	Berkeley S1 M	EC-SC-IC 63	Arena with objects	2x10 mins	Object zone entries: EC>SC Object manipulation diversity: EC>SC/IC
Renner 1987	S1 M	EC-IC 120	Arena with objs Familiar vs Unfamiliar	2x10 mins	Bout length EC>IC Total Interaction Time: EC>IC No of Diff Behavs EC>IC Use of Paws EC>IC
Renner 1988	Sprague Dawley M	EC-IC 120	Arena containing obstruction box and objects	2x10 mins 2 days	Time with Box EC>IC Investig/n of Box EC>IC (day one only)
			Arena with Box and Predator Car	2x3mins	Time to Escape IC>EC
	Sprague Dawley M	EC-IC 120	Arena Box and Predator Car	2x3 mins	Time to Escape IC>EC
Widman and Rosellini 1990	Sprague Dawley M	Ec-IC 2 hrs per day 75	Arena with Objects	10 mins	Time in obj Contact EC>IC No of Bouts with Objs EC>IC Latency to Initiate Bouts IC>EC Bout Diversity EC>IC

Table 2:22 continued

Renner 1984; Renner and Rosenzweig 1986; Renner 1987; 1988; Widman and Rosellini 1990) the Greek Cross (Klippel 1978; Ferchmin et al 1980) mazes (McCall et al 1969) the Berlyne box (Sahakian et al 1977) and in the animal's home cage (File 1978). Of these 24 studies, outlined in more detail in Table 2:22, 18 have included objects, extending the concept of novelty to include both the environment and its contents.

Considering first those studies that have examined the effects of novelty of environment alone, several interesting points have emerged. With respect to the nature of the environment itself, it has been argued that research directed towards the problems of reactions to novelty should attend to the stimulus characteristics differentiating the novel environment from the environment in which the subject has been adapted (Woods 1962). In particular it has been suggested that an increase in the complexity of the environment relative to the home cage, rather than the effects of novelty or change per se, results in an increase in exploration (Woods 1962; Woods and Davidson 1964). A second and related idea is that of Zimbardo and Montgomery (1957) who suggest that differences between enriched and deprived animals may be partially a function of the degree of novelty the exploratory apparatus represents for the subject. For example, they suggest that if the apparatus is complex it may represent an appropriate level of novelty for the enriched animal, but be too novel, maybe even fear provoking, for the deprived organism. A more detailed statement of such a relationship is the discrepancy hypothesis (Dember and Earl 1957) which suggests that exploration should be an increasing function of the degree of discrepancy between a familiar standard and a new stimulus up to a point, after which greater magnitudes of discrepancy recruit less and less exploration.

However, other factors may also influence the effects novelty of environment on differentially housed subjects. For example, enriched animals appear to be influenced by stimuli external to the test environment (Brown 1968; Forgays and Forgays 1952; Hymovitch 1952). Indeed McCall et al (1969) have reported that their "results do not support the proposition that the novelty or complexity of the exploration chamber interacts directly with rearing condition" (p759), but

they did find extra-field cues and in particular the presence of the animals' caretaker, interacted with rearing condition. Secondly, there is some indication that the effects of differential rearing manifest themselves in the rate of habituation to a novel stimulus complex (Ehrlich 1959). For example in a paper by Ferchmin et al (1980) the level of complexity during rearing was found to determine the optimum degree of stimulation which was sought by the animal, however, this was mediated by day of testing (cf Rose et al 1985). Thirdly, differences between rearing conditions seem to be a function of whether the exploration is forced or free (Welker 1957, Lore and Levowitz 1966).

Whether it is concluded complexity of environment and the other factors outlined above produce specific responses in EC, IC and SC animals, also depends on the measurement of exploration employed, and the method and onset of isolation (Sahakian et al 1977; Klippel 1978). In particular, certain measures of exploration, such as latency to emerge, are confounded with emotional factors (see next section), whereas others are confounded with locomotor activity (Welker 1957; Leyland et al 1976). In an attempt to separate out exploration and locomotor activity, Sahakian et al 1977 employed a design in which preference for a novel over a familiar environment was measured in a setting that permitted an independant measure of activity. The main finding was that restricted rats showed an enhanced preference for novelty, supplementing the finding of Ehrlich (1961) that rats reared in restricted environments showed higher levels of operant responding for stimulus change, when compared with controls. If activity is employed as the dependant variable in a novel environment, isolates are also found to be more active than their controls (Syme 1973), although this appears to be age dependant. Interestingly, McCall et al (1969) have pointed out that differences in exploration may not be revealed in measures such as squares traversed or time spent exploring, but rather in terms of object contact and exploration of specified portions of the field. In the remainder of this section, studies that have concentrated on these variables will be reviewed in more detail.

The first study of the effects of differential environments on object contact (Lore and Levowitz

1966) reported that socially isolated rats spent more time in contact with objects in an open field than their enriched counterparts. Typical of the confusion in this area of research, however, in 1969 McCall, Lester and Dolan failed to find any differences between their groups. In particular, they reported no significant differences between EC and IC animals in terms of the length of object contact or in the differences between time spent in contact with movable versus immovable objects. However, they did find differences in styles of exploration, reflecting a more systematic exploration on the part of the enriched subjects. Generally, EC animals "moved slowly about the field looking both inside and outside the apparatus (extra field exploration) and cautiously approached the objects contacting them with their paws" (p 758). In contrast the deprived subjects "appeared to dash about the field without attending to extra field cues and often bumping into the objects" (p758).

Stylistic differences have also been reported by Renner and his colleagues (Renner 1984; 1987; 1988; Renner and Rosenzweig 1986; Widman and Rosellini 1990), EC's demonstrating considerable diversity in the manner in which they manipulated objects. As these studies share similar methodologies and comparison groups (namely EC-IC), they will be reviewed together before considering the qualitative differences that have emerged in studies comparing SC and IC animals.

The earliest study in this series (Renner 1984) found that rats from enriched and impoverished environments displayed different strategies of exploration, although the groups did not differ in "overall willingness to explore or interact with new stimuli" (p3109-B). In particular, EC animals used more diverse forms of interaction than their IC littermates, but only with objects small enough to be manipulated by the subjects. This work was further detailed in Renner and Rosenzweig (1986) [6] where age of subjects was reported. In these two papers exploration was reported in juvenile rats (30-60 days of age) a time when these animals have a high rate of activity anyway. In a follow-up study (Renner 1987) animals' behaviour was investigated

[6] Renner (1984) is a brief resume of a Doctoral Dissertation published in more detail by Renner and Rosenzweig 1986).

in adulthood (120 days). Animals were housed in differential environments from 90-120 days and then individually tested in an adapted hemioctogon arena containing four objects. These were chosen according to whether they were familiar or unfamiliar to the subject and whether they could be manipulated or not. Subjects were videotaped for two ten-minute sessions on two seperate days and both activities not related to the objects (locomotion, grooming) and behaviours associated with interacting with the objects were recorded. Isolates, as would be predicted, spent more time in the start box than their EC counterparts and reared more in the arena. The most interesting differences between the groups, however, emerged in their object interaction behaviour. All the subjects interacted more with non-manipulable objects, with little evidence that the novelty of the object was an important factor. Mean bout length, however, was higher for EC than IC animals, as was total interaction time. In addition EC animals displayed a greater diversity of behaviours towards objects than the IC animals, both groups preferring manipulable objects to static ones. More specifically, EC animals were more likely to use their paws and to climb on objects than their IC counterparts. Coupled with the previous experiments this study "makes it apparent that the quality and the quantity of exploratory behaviour and its character are separable properties" (Renner 1987 p97). This in turn "raises serious questions about the practice of measuring and discussing exploration as if it were a unitary phenomenon" (p97).

One question which remains to be answered is the functional significance of these differential effects of enrichment and impoverishment on object interaction. Renner (1987) has speculated that "some aspects of exploration may have important consequences for animal information gathering and problem solving" (p97). It may well be that EC animals are simply more inquisitive (or as Renner says have "a broader range of inquisitive behaviours" p97). However, it might also be that this is an environmentally-induced adaption, a greater range of exploratory abilities allowing the EC animal more opportunity to investigate and learn about its environment.

As well as age at which environmental experience and testing occurs Widman and Rosellini

(1990) have recently considered a second temporal pattern in these effects, namely the amount of experience itself. In their study, employing a procedure based on Renner's work, they exposed rats to enrichment for two hours a day for 30 days. When tested EC animals were found to demonstrate a higher number of bouts with objects than their IC counterparts, enrichment also decreasing the mean latency to initiate a bout although this latter finding was not statistically significant. In addition, a qualitative analysis of behaviour revealed that the EC animal employed a greater diversity of behaviours towards the objects, all of these findings being reminiscent of Renner's earlier work. Again these authors question the functional significance of this behaviour, highlighting the neuroanatomical and neurochemical changes that have been noted in animals exposed to enrichment for two hours a day or less (see chapter one). To date, however, no studies have explored the relationship between brain anatomy and exploration.

The final study to examine EC-IC differences in reaction to novel objects is an unusual escape procedure, devised with some ingenuity by Renner (1988). After 30 days of differential housing starting at 90 days animals were habituated to a test arena for two ten-minute sessions (on consecutive nights). Based on an open field, this arena had a 10cm diameter hole cut in its center, suspended under which was a plastic cage. A wooden box was placed in the center of the arena over the escape hole, with a ramp inside the box linking the floor of the arena to a 10cm hole in the top of the box. All subjects could climb onto the box and down through the hole onto the arena floor and then down into the escape cage. On the third night, animals were re-introduced to the arena and after three minutes a "simulated predator", in fact a radio-controlled car was introduced into the arena, controlled in such a way by the experimenter as to chase the animal (trying not to make contact with it) until it had either escaped or 180 seconds had elapsed. Behaviours under challenge were video-taped and analysis revealed that on first noticing the predator IC animals were more likely to jump, whilst time to escape under challenge was significantly lower in the EC animals [7]. In a second experiment in this study, animals were not given any time to habituate to the environment and this time none of the IC animals

[7] There was a high degree of variance in these behaviours.

were able to escape from the car. The implications of this latter experiment are interesting, as it suggests that being enriched does have a marked advantage in situations where quick adaption to the environment is of paramount importance. The qualitatively different exploratory behaviours of EC and IC animals may well create in the EC animals an opportunity to learn more quickly from their environment and profit from this in a dangerous situation [8].

Moving on now to SC-IC comparisons, qualitative differences have also been observed between these groups (Einon and Morgan 1976), however, unlike Lore and Levowitz (1966), these authors found that rats reared in social isolation contacted fewer novel objects in an open field than their socially housed littermates, over a seven minute period. Isolates also contacted a smaller variety of objects and contacted them in different ways. In particular, social animals were found to show more manipulatory behaviour than isolates reminiscent of the enriched animals behaviour. In a second experiment in this study, Einon and Morgan (1976) examined habituation of object contact over thirty minutes and found that initially the socially reared animals contacted more objects, but the number of contacts they made declined quite rapidly with time. Isolates on the other hand, contacted fewer objects initially, and although they showed some decrement with time, this occured more slowly. This difference in the rate of habituation may well explain why Lore and Levowitz (1966) found that isolated animals contacted more objects, as they employed a fifteen-minute behavioural sample, quite a long trial length.

The finding that isolated animals differ in the rate at which they habituate is in agreement with the findings that these same animals show a slower decline in activity over time in an object free open field (Einon, Morgan and Sahakian 1975). Indeed, Einon and Morgan (1976) point out that "the fact that isolated animals are slower to habituate in both a simple and complex environment argues against Zimbardo and Montgomery's (1957) suggestion that the difference between deprived and enriched animals may be partially a function of the degree of novelty of the exploratory field" (p418). They suggest "that the differences between socially reared and isolated

[8] Survival ability in differentially housed animals has been tested by Roeder, Chetcuti and Will (1980) under conditions of pole-cat predation, EC animals having a survival advantage over their IC littermates after day 15 after onset of predation. Overall survival rates, however, were not statistically significant.

animals in a novel environment are not profitably described in terms of a simple "exploration" hypothesis. Rather the differences seem to depend upon rate of response training and upon the precise details of the way in which the animals expose themselves to novel stimulation" (p419).

The way in which restricted cats react to novel stimulation would appear to support this contention. For example, Konrad and Bagshaw (1970) have reported that cat behaviour emerges in a complex novel environment in a particular sequence, namely approach, explore and play. When presented with a strange room, both restricted and control cats displayed these sequences of behaviours, but differed in the *rate* of development of the sequence.

As well as differences in styles of exploration (McCall et al 1969; Konrad and Bagshaw 1970; Einon and Morgan 1976) and rate of habituation (Einon and Morgan 1976; Sahakian, Robbins and Iversen 1977) isolates have also been found to have a significantly higher frequency of bouts of exploration (Sahakian, Robbins and Iversen 1977), initiating exploratory sequences of behaviour more often than controls, but also terminating them sooner. Furthermore, these effects appear to be age dependant (Einon and Morgan 1977) with a slow decline in object contact resulting from isolation before 45 days of age. Subsequent social housing does not reverse this effect, although exposure to social contacts for one hour a day during this "critical period" does alleviate some of the differences between IC-SC animals (Einon, Morgan and Kibbler 1978). These findings have led the researchers to conclude that "normal development in the rat may depend upon the flexibility of behaviour encouraged by the early social situation" (p214), in that the isolated rat lacks the experience of rapid attenuation of roles and behaviour patterns that are characteristic of social interaction in infancy. Moreover, these effects do not appear to be species specific, although the relationship between object investigation and social rearing is more complex in the ferret than it is in the rat (Chivers and Einon 1982).

In summary then, it can be seen that differential housing produces animals with distinctive behavioural profiles with respect to their reaction to novelty. Procedural differences in the studies renders comparison difficult, however, from the above, it can be seen that isolates are slower to

habituate to novelty, making less object contacts initially, but are more likely to indulge in higher frequencies of albeit shorter bouts of object contact, as time goes by. Socially housed animals are more purposeful in their styles of contact and are more receptive to extra field cues, whilst EC animals appear to be armed with a behavioural repertoire of exploratory behaviours which confer upon these animals some functional advantages. Age of experience, trial length and type of test apparatus also produce significant effects which may explain the diverse results reported in Table 2:22.

5: Emergence Procedures

Within the EC-SC-IC literature, parameters of general activity have typically been varieties of motor behaviour. According to Walsh and Cummins (1976), however, activity can also be scored by its absence and parameters in this instance are identified by the terms "latency" and "freezing". Latency is measured by the time taken from the start of a trial to the occurrence of a certain type of behaviour, whereas freezing is defined as the absence of activity. One procedure that incorporates both of these parameters is the *emergence or timidity test* (Denenberg 1967), where the dependant variable is typically latency to emerge from either the home cage (Ader and Friedman 1964; Einon et al 1975; Einon and Tye 1975) or from a start box (Moyer and Korn 1965; Gill et al 1966; Renner 1987) or from a specialised emergence tube or box (Einon et al 1981; Curry 1987). Freezing, or failure to emerge is also taken into account in this apparatus by incorporating a cut off point ranging from three minutes (Einon and Morgan 1977) to 15 minutes (Ader and Friedman 1964), with any animal failing to emerge in this time being allocated a maximum score.

In this section, the effects of differential environments on an animal's response to the emergence test will be reviewed. As can be seen from Table 2:23, there have been 19 studies incorporating at least 30 experiments in this area of research, examining a range of dependant variables including latency to emerge, latency to the first appearance of any portion of the animal, total time part

AUTHOR	COMP	STRAIN SEX	TEST AGE	TEST ENVIRON	TEST PROCEDURE	FINDINGS
Ader and Friedman 1964	SC-IC	Sprague Dawley M	106 86	Home Cage On Table	Latency To Emerge	N/S IC>SC
Moyer and Korn 1965	SC-IC	Albino M	111	Start Box	Latency To Emerge	IC>SC
Lore and Levowitz 1966	EC-SC-IC	Wistar M	86-88	Home Cage	Partial Emergence Time Part Of Rat Outside Cage No Of Animals To Emerge	N/S Group Differences EC>SC/IC
Gill et al 1966	EC-IC	Long Evans M	81	Start Box Jump Stand	Latency To Emerge	IC>EC
Konrad and Bagshaw 1970	EC-IC	Cats F	15 Months	Start Cage Novel Environ	Latency To Emerge	IC>EC
Levitsky and Barnes 1972	EC-IC	Rats M	No Detail	Entry Into Novel Environ	Percent Of Animals Entering	IC>EC
Morgan 1973	EC-IC	Hooded Lister F	104	Emergence Into Into O/F	Latency To Emerge	IC>EC
Einon et al 1975	SC-IC	Hooded Lister M F	15 25 45 45	Emergence Into O/F Home Cage	Latency To Emerge	N/S N/S IC>SC IC>SC
Einon and Tye 1975	SC-IC SC-IC	Hooded Lister M F M F	100 110 60	Emergence Tube Home Cage Emergence Tube	Latency To Emerge	IC>SC F>M Saline>Drugs N/S N/S N/S
Einon and Morgan 1977	SC-IC (GG/IG vs II/GI)	Hooded Lister M F	45 90 180	Emergence Tube	Latency To Emerge	GI/II>others GI/II>others GI/II>others
Klippel 1978	EC-SC-IC	Mus Musculus M F	63 77	Beaker Into O/F	Latency To Emerge	N/S
File 1978	SC-IC	Hooded Lister M	85 To 95	Hole Board	No Of and Duration Head Dips	SC>IC SC>IC
Joseph and Gallagher 1980	EC-IC	Zivic Miller M F	84	Emergence Into O/F	Latency To Emerge	N/S
Einon et al 1981	SC-PI-IC	Hooded Lister M Mus Musculus M F	46 70	Emergence Tube	Latency To Emerge	Rats N/S Mice: IC/PI>SC Rats: N/S Mice: N/S
Benton and Brain 1981	SC-IC	TO Mice F	112-301	Tube In In Cage	Latency To Emerge	IC>SC
Warren et al 1982	EC-IC	Mus Musculus M	over 600	Emergence Into O/F	Latency To Emerge	N/S
Holson 1986	SEC-IC	Long Evans M	120 approx	From Runway into O/F or box	No of Entries N/S	
Curry 1987	EC-IC	Hooded Lister M	70	Emergence Into O/F	Latency To Emerge	N/S
Renner 1987	EC-IC	S1 M	120	Into Arena	Latency to Emerge	IC>EC

Table 2:23 details those studies employing emergence procedures.

or whole of the animal is outside the emergence apparatus and number or percentage of animals from the different groups to leave the apparatus. Unfortunately, as with the open field procedure, these dependant variables have been differentially interpreted as evidence of several underlying constructs including emotionality (Moyer and Korn 1965) and exploration (Lore and Levowitz 1966). Unlike, the open field procedure, however, there is a consistency among the results in this area of research. Generally, isolates have been found to have a greater latency to emerge than their socially housed counterparts, although in some experiments this tendency did not attain statistical significance (Einon and Tye 1975; Curry 1987). Furthermore, this finding is not specific to rats, with mice (Einon et al 1981) and cats (Konrad and Bagshaw 1970) being similarly affected.

Early interpretations of these findings suggested that individually housed animals were more emotional than their socially housed counterparts (Ader and Friedman 1964; Moyer and Korn 1965), although as Hahn (1965) has pointed out, the term emotionality must be used with caution. Alternatively, these results were considered to demonstrate low exploration in restricted animals (Lore and Levowitz 1966), however, as Morgan (1973) points out, the weight of evidence does not support either of these explanations, although he does not offer any alternative, commenting simply that the reason for IC animals being slower to emerge 'is not known" (p439). More recently, in a study testing animals for emergence at 15, 25 and 45 days of age (Einon et al 1975), isolates were found to differ from socially housed animals only in the last age group and these results were interpreted as being "consistent with the view that social isolation results in an abnormal developmental process, rather than a freezing of development at one of its normal stages" (p 558). The effects of isolation on emergence, can however, be reversed by subsequent social housing (Einon and Morgan 1977; Klippel 1978), and it has been suggested that play behaviour may well be implicated in mediating this reversal (Einon et al 1981). Interestingly, isolating socially housed animals at 600 days of age, has no effect on their latency to enter an open field (Warren et al 1982), corroborating Einon and Morgan's (1977) suggestion of a critical period for social isolation in the rat. Overall, however, results in this area of research suggest

that isolation produces an animal with an increased latency to emerge, when compared with socially reared counterparts.

c) Summary of General Activity Findings

Within the EC/IC literature there have been numerous studies of general activity, producing often conflicting results. These reflect the wide range of test situations employed, the lack of consensus regarding the precise definition of the term "general activity" and its underlying causes. In this review Lamden's (1985) division of general activity into its two distinct components, basal activity and reactivity, was adopted and from the experiments investigating these components a clearer pattern of the impact of early experience has emerged.

Considering those few studies that have investigated basal activity first, there is a tendency for IC animals to be more active in their home cage than their EC counterparts[9] (Lamden 1985). Indeed, Baenninger (1967) has noted increased exploratory behaviour, locomotion, pawing and tail manipulation in these animals when compared with SC controls. However, these experiments are all fraught with methodological problems, as a consequence of which true baseline activity levels of EC, SC and IC animals remain to be elucidated.

With respect to reactivity, however, from the various test situations employed, several key distinctions have emerged between the differentially housed animals' reactivity profiles. Typically IC animals are more active than either their SC or EC counterparts, but this activity pattern only emerges if animals are tested over a period of time. Furthermore activity levels are dependant on the complexity of the test environment relative to that of the animal's home cage.

IC animals have been found to maintain higher levels of activity in the open field over days (Woods et al 1960; Levitsky and Barnes 1972; Fessler and Beatty 1976; Domjan et al 1977; Lamden 1985; Dell and Rose 1987; Curry 1987) and to demonstrate a perseverative tendency

[9] By home cage, Lamden means animals measured in the individual cages they are maintained in following differential rearing.

to explore in the Hebb-Williams maze (Joseph and Gallagher 1980) compared to their socially enriched counterparts. These latter animals, although initially reactive tend to reduce their activity over days. These pattern of responding have been interpreted in various ways, but typically impoverishment is seen as being detrimental to its inhabitants. In particular, IC animals are seen as having an abnormal developmental pattern (Einon et al 1975) characterised by a general disturbance of inhibitory mechanisms and/or hyperarousability (Einon and Morgan 1978). Enrichment, on the other hand, is seen as reducing the novelty value of the test environment thus reducing the reactivity levels of its incumbants.

The impact of the differential environments seems to produce animals whose early experiences interact with the test situation, such that the complexity of the test relative to the home cage environment is a crucial factor in determining the level of activity initially displayed. Impoverishment, in addition, produces an animal with a slower rate of habituation to stimulation. This "habituation" hypothesis, characterised by a "more environmentally reactive animal which habituates more slowly" (Lore 1968 p571) can also be seen in the activity monitor data. Generally IC animals are significantly more active in this apparatus than their comparison group.

However, the novelty of the environment is also an important consideration. It has been suggested that animals raised in "complex environments develop perceptual skills or strategies for processing information" whilst animals reared in "restricted environments are unable to handle the discrepancy between the limited range of their early experience with the typical variety and complexity of test environments" (Kessen 1968 p396). This "discrepancy" hypothesis has been applied to the finding that EC animals are more active in an activity wheel than their less enriched comparison groups. The activity wheel comprises both a "home cage" and an attatched running wheel. If the running wheel is seen as a novel environment then it is not unexpected that the animal with the larger discrepancy between test environment and rearing condition would spend more time in the home cage habituating to it, before venturing out into the activity wheel and clocking up a high score there.

Indeed, the data examining emergence into a novel environment would tend to support this view, IC animals typically taking longer to emerge than either their SC or EC counterparts. Initially it was suggested that IC animals were more emotional than their socially housed counterparts (Ader and Friedman 1964; Moyer and Korn 1965) or demonstrated lower exploration (Lore and Levowitz 1966). However, neither of these explanations are satisfactory when one considers other measures of emotionality and exploration.

For example, one of the dependant variables in the open field, defecation, which has been factorially associated with emotionality, does not highlight the IC animal as emotional. Indeed, if anything, it is the SC animal which defecates the most in this test situation. Furthermore, if the IC animals were more exploratory, then why should higher levels of exploration be noted in enriched animals by Zimbardo and Montgomery (1957) in the Y maze? Perhaps the most reasonable suggestion to date is that impoverishment can be seen as altering the animal's developmental process (Einon et al 1975) resulting in hyperarousability. Furthermore, the effects of isolation on emergence can be ameliorated by subsequent social housing, with access to conspecifics with which to play being implicated in mediating this reversal of behaviour.

Perhaps the most complete behavioural profile of differentially reared animals' reactivity, however, has emerged from the study of their reactions to novelty. First of all it appears that it is the increase in complexity of the environment relative to the home cage rather than novelty alone which is the most important factor to be taken into consideration (Woods 1962; Woods and Davidson 1964) reminscent of the discrepancy hypothesis outlined above. However, given this fact, of particular relevance to this summary are the behavioural profiles of animals exposed to differential environments when introduced to a novel environment.

EC animals have been found to be more influenced by extra-field cues, whilst IC animals show a preference for novelty over activity (Sahakian et al 1977) suggesting that they are stimulus seeking. With objects introduced into the novel environment, IC animals tend to dash about almost accidentally bumping into the objects, contact less objects than their EC counterparts, but

having contacted the object, indulge in a higher frequency of bouts of interaction and maintain higher levels of activity over time. If startled by a "predator car" these animals react by jumping and failure to escape.

A completely different profile emerges for the EC animal. When encountering a novel object this animal systematically explores it, cautiously manipulating it, employing a diversity in manner of interaction with the object. EC animals also have longer bout lengths and total interaction time than their IC counterparts, climb on the objects, use their paws and show a marked adaption to their environment considerably more quickly than their isolated peers. Interestingly these animals also habituate more quickly reducing their interactions over time.

These behavioural patterns suggest that enrichment has an adaptive function aiding survival. In particular a cautious approach initially, a thorough investigation followed by habituation to a novel object seems to be a more "thoughtful" way of dealing with the unknown when compared with the behaviour of the isolated animal. Having charged around encountering objects, of which only a few are selected for further manipulation, this animal continues to maintain a high level of interaction, perhaps stimulus seeking. Several explanations for this behaviour could be proffered, including maladaptive development of exploration and play sequences (Konrad and Bagshaw 1970) in the IC animal, coupled with poor inhibition of inappropriate responses. Additionally, enrichment can be seen as ameliorative, producing animals with enhanced problem solving abilities and information processing (West and Greenough 1972) which they can adapt to any novel situation and employ the appropriate reactions.

Whatever the causes, what does appear to be true is that exposing animals to EC, SC or IC produces very different patterns in their general activity levels irrespective of whether these are measured in the open field, emergence procedures, activity monitors, novel environments or mazes.

2:3:2 PERCEPTUAL ABILITIES

Within the EC-SC-IC literature, interest in the effects of differential environments on an animal's perceptual abilities has often emerged as a by-product of examining their performance in various learning paradigms. Indeed, as Lamden (1985) points out "any attempt to describe those characteristics of enriched and impoverished animals which may potentially influence their performance in subsequent learning situations must necessarily include an attempt to assess those aspects of sensory function which are commonly implicated in such performance" (p233). In this section two such sensory capacities are reviewed, namely depth perception and perception of noxious stimuli, as these are the procedures which have been most employed in the EC-IC literature.

a) Depth Perception

Early interest in the enriched rats' use of depth or distance perception stemmed from reports that these animals were using extra-maze cues as a problem solving strategy in the Hebb-Williams paradigm (Hymovitch 1952; Forgays and Forgays 1952). Furthermore, caretaker effects such as those reported by McCall et al (1969) suggested that EC animals were using distance cues to identify the caretaker outside the test apparatus. These experiments support the notion that EC rats have either more highly developed depth/distance perception, or at least make greater use of depth and distance cues. Intuitively, such a conclusion is unsurprising, since the EC environment must afford its occupants greater experience of observing distance (Lamden 1985) and actively interacting with depths when climbing walls and ramps, than their IC counterparts.

Eichengreen, Coren and Nachmias (1966) were the first researchers to test EC-IC animals' depth perception, using the visual cliff apparatus first employed by Walk, Gibson and Tighe (1957). Animals were differentially reared from 10-22 days of age and were either tested normally or were monocularily occluded. When tested binocularily, both groups preferred the shallow side, but only EC animals maintained this preference under monocular occlusion. These authors suggested

on the basis of their results, that depth perception in the rat is influenced by experiential factors. However, earlier studies had shown that binocular depth perception in the rat does not require prior visual experience (Walk, Gibson and Tighe 1957). On the other hand, investigators have asserted that even in binocular tests, performance on the visual cliff depends on essentially monocular cues (Trychin and Walk 1964). Eichengreen et al (1966) contend that their results appear to reopen the question of what cues are employed in the visual cliff, a question that has been the subject of much of the investigation of visual cliff behaviour.

In their 1961 monograph, Walk and Gibson suggested that the stimuli involved in depth perception are visual, tactual, vestibular and kinaesthetic, with changes in stimulation arousing reflex postural reactions and a feeling of fear (p1). Furthermore, according to an earlier report (Russell 1932) vision is the most important sensory avenue involved in identification of depth in the rat. Some of the possible factors involved in *visual* depth discrimination that have been considered include accomodation, convergence, binocular parallax, monocular parallax, aerial perspective (intensity, texture) and geometric perspective (Russell 1932). Of these, two factors have emerged as being particularily important for depth perception in the rat, namely pattern density and motion parallax (Walk and Gibson 1961). For visual detection of a drop-off or edge, light to the animal's eyes must provide information to differentiate the drop-off from the surface on which the animal stands, it must provide stimulation for an edge and ideally for gradations of depth below the edge. Such cues are engendered if two surfaces at different heights are textured or patterned (Gibson 1950; 1958).

The same situation provides a second kind of differential stimulation for depth discrimination if the animal moves. Head movements or a change in position as the animal looks, will produce motion parallax. The velocity of angular motion of texture elements at the line of the optic array corresponding to the edge of the platform will be different from the velocity of elements of the surface below (Walk and Gibson 1961 p2). Motion parallax (differential velocity of elements in the array) will increase as the drop increases. There will then be a velocity difference between

the ground and the surface below, which will characterise the relative depth of the surface below. This velocity difference produced by the animal's own movement is potentially a highly effective kind of information about the relative depth downwards of a surface (Walk and Gibson 1961).

The question of which of these cues is of greater importance has been the subject of considerable investigation. In their review, Walk and Gibson (1961) stated that on the whole, the evidence supported the conclusion that motion parallax was the more effective cue (p30). Since then the topic has become more complex, following DeHardt's (1969) observation that certain textures are aversive to rats. Following replications of these findings Walk and Walters (1974) concluded that for the rat at least, the visual choice of deep versus shallow side is made more by texture than by motion parallax. Gibson and Walk (1960), however, have suggested that of the two visual cues implicated, only motion parallax was an innate cue for depth perception, whilst responses to differential pattern density might be learned later.

Whatever the specific depth cues employed by animals in the visual cliff tests, it is accepted that there are other factors to be taken into account when explaining overall behaviour (Lamden 1985). For example, Routtenberg and Glickman (1964) have suggested that both visual activity, emotionality and exploratory tendencies may regulate measures of visual cliff behaviour. Futhermore, according to Tees and Midgely (1978) the "ontogeny of depth perception in the rat involves the collaboration of a number of factors, including innate, maturational and experiential components" (p774).

Of particular interest to the present research is the influence of the latter component, experience, on an animal's depth perception. Most of the literature has concentrated on *visual* experience, with animals exposed to dark rearing (Walk and Gibson 1961; Walk 1978). However, there are three studies that have specifically considered the effects of environmental enrichment on an animal's depth perception, one of which (Eichengreen et al 1966) has already been described above. The other two studies (Lamden 1985; Curry 1987) will be outlined below.

Using animals housed in enriched or impoverished environments for thirty days following weaning,

Lamden (1985) tested each animal once at each of four visual cliff depths (0, 15, 30 and 45 cm) on consecutive days. Two dependant variables were measured, latency to descend and preferred side (shallow or deep) and significiant EC/IC differences were found. In particular, at the 15cm depth all 15 EC animals and 9 out of 15 IC animals chose the shallow side, exhibiting depth perception and EC animals typically took longer to decide which side to choose, as measured by latency to descend. No significant differences in side preference emerged in the other three cliff depths. Obviously none would be expected at 0cm's depth, it being the equivalent of the shallow side, however, the finding that 30cm and 45cm cliff depths produced no significant differences between the groups was interesting. Lamden suggested that perhaps these depths were beyond the visual capacities of the rat. With respect to the latency to descend measure, at the 30cm cliff depth, however, the EC deep side latencies were significantly larger than the corresponding IC latencies, at least suggesting the possibility of EC/IC depth perception differences in favour of the EC animal at this depth also. Following a similar procedure, but only using one depth (approx 30 cm), Curry (1987) found no significant side preferences in his EC/IC groups. Unlike Lamden's findings, however, his IC animals took longer to decide which side to descend onto. This finding leads one to question what latency to descend is actually measuring. For example, Lamden has suggested it reflects decision making in the animal, however, it has been found that IC animals have longer latencies to emerge (see earlier section) and Curry's findings may well be replicating this emergence behaviour. With respect to depth perception as measured by side chosen, however, both Eichengreen et al (1966) and Lamden (1985)'s findings suggest that EC animals have a more highly developed depth perception, or at least are more inclined to use depth cues, than their IC counterparts.

b) Perception of Noxious Stimuli

In an earlier section of this chapter (Section 2:2:4) the effects of differential environments on avoidance learning were reviewed. Inherent in this paradigm is the notion that an animal will

respond in such a way as to prevent the occurence of an aversive reinforcer. Perception of this reinforcer, according to Hebb (1949) involves a complex process in which a major role is played by all kinds of earlier perceptual learning, including both specific and non-specific experience involving all the senses. If, as this theory suggests, early experience plays an important role in perceiving and responding to pain, differences observed in EC-SC-IC avoidance learning tasks may well reflect differences in their perception of noxious stimuli, rather than differences in learning per se. In this section studies that have specifically examined EC-SC-IC animals' perception of noxious stimuli will be examined and implications for avoidance learning paradigms discussed.

One of the first studies designed to investigate the influence of early experience on the perception of pain was that of Melzack and Scott (1957), in which the pain avoidance behaviour of isolation reared terriers was compared with pet reared littermates. The restricted subjects exhibited gross deficits in avoiding severe electric shock in two avoidance conditioning tasks, lighted matches thrust at their noses and needles jabbed into their flanks. The outstanding feature of the behaviour of the restricted dogs was "their inability to respond adaptively and intelligently to the variety of stimuli which were presented to them" (p159). With respect to the shocks, Melzack and Scott report that there is little doubt that the restricted animals felt the stimulus, as "their disturbance (by it) was marked and unmistakable" (p159). Futhermore, when burned or jabbed, restricted subjects made no attempt to get away from the experimenter. In all the experimental situations, these animals appeared to be incapable of performing "the proper avoidance responses which would have prevented further stimulation" (p159). The inability of the restricted dogs to cope intelligently with noxious stimuli, however, could not be attributed to inadequate response mechanisms alone. As Melzack and Scott point out "their reflexive jerks and movements during pin-prick and contact with fire suggest that they may have felt something during stimulation; but the lack of any observable emotional disturbance apart from these reflex movements... indicates that their *perception* (sic) of the event was highly abnormal in comparison with the behaviour of the normally reared control dogs" (p159-160). Melzack and Scott concluded that early percep-

tual experience can strongly influence the development of both an adequate repertoire of pain avoidance responses and the actual capacity to perceive pain normally.

One criticism of this study was that Melzack and Scott administered their pain avoidance tests in totally unfamiliar settings (Lore 1969). Indeed, there is some research that suggests that normal perceptual development requires only minimal sensory stimulation and that many of the behavioural deficits observed in experientially deprived animals are the result of an exaggerated fear reaction elicited by the testing situation/procedure (Fuller and Clark 1966a; 1966b). Postulating that isolation reared animals which appear to be insensitive to pain when tested under generally stressful conditions may behave very differently when the same tests are conducted in a non-stressful environment, Lore (1969) exposed EC-IC rats to a lighted candle in both the home cage and a novel test cage for five minute periods. All subjects readily investigated the candle, but the IC animals made significantly more nose-flame contacts in the novel test cage. However, in the home cage test, the pain avoidance behaviour of the restricted rats was entirely comparable to that of subjects exposed to an enriched environment. These results indicate that increased emotional reactivity can account for IC animals' apparent perceptual insensitivity to pain and suggest that Melzack and Scott's "perceptual deficit" interpretation of their findings may be unwarranted, because they failed to control for differences in the emotional reactivity of their subjects.

More recently, Fessler and Beatty (1976) extended the concept of differential environments to include a standard housing condition, as well as enriched and impoverished conditions and measured shock thresholds over ten trials at each of thirteen shock levels ranging from 0.005-0.80mA. Thresholds for flinch, shuffle and jump responses, which were defined as the lowest shock levels at which the animal exhibited the response on 50% of the trials, were computed for each subject. Interestingly, animals raised in an enriched environment were found to have significantly higher thresholds on each of the measures, than animals in the other groups, a result that was unpredicted given that the earlier work (Melzack and Scott 1957; Lore 1969) had found social

and sensory restriction profoundly attenuated the response to painful stimuli.

One criticism of this study (Lamden 1985) was that Fessler and Beatty did not take into account Pare's (1969) finding that shock sensitivity was directly related to body weight. Indeed, in her study which was designed to observe EC/IC responsivity to shock in an actual behavioural test situation, Lamden (1985) attributed her finding of EC/IC differences at low shock levels to both differences in the groups' body weights and to differences in their motor abilities. In particular, she found that the lighter EC animals were faster to leave the start box and spent less time in the alley of a runway apparatus than their heavier IC counterparts. Furthermore, she found that the EC/IC difference in responsivity to shock was greatest when the shock level was 0.1mA and 0.2mA. Indeed, at the lower of these shock levels there was little discernable evidence that the majority of the IC group were feeling the shock at all, as they did not display any of the overt signs of shock sensitivity (flinch, jump or vocalisation) and they took much longer to exit from the start box and run to the goal box. However, at higher and more painful shock levels, the the differential between the two groups fell to such an extent that there was little discernable difference between the performances of the EC and IC animals.

The implication of this work is that IC animals are not as sensitive to low intensities of shock, as their EC counterparts, but that both groups are equally sensitive at higher intensities. These findings contradict those of Fessler and Beatty (1976) and of particular importance to this present review, have obvious ramifications for situations where the learning performance of EC/IC rats involves shock motivation. As Lamden points out "unless the shock level in such situations is set at a level higher than 0.2mA, differences in performance that have previously been attributed to a deficiency in the learning capacity of the IC animal may actually be due to differential sensitivity to shock for which the EC/IC differences in body weight are largely responsible. In other words, the heavier IC animals may simply not feel the lower intensities of shock and would therefore not be motivated to escape from it" (p 318-319).

2:3:3 MOTOR ABILITIES

According to Lamden (1985), motor skills have been the focus of a limited interest within the published EC/IC literature, with this interest being divided between two issues. The first concerns the extent to which the more varied requirements of enriched environments can account for subsequent EC/IC differences in both brain and behaviour (Rosenzweig and Bennett 1976), whereas the second concerns the differences between the *motor capacities* per se of EC and IC animals, following environmental manipulation. It is the data relevant to the latter issue which are of interest in this present review.

One of the earliest investigations of the motor capacities of differentially housed animals was that of Morgan (1973), who designed two tests that required complex motor skills of his EC, IC and SC animals. In the first, rats were trained to run through an alley for food reward and subsequently found the alley blocked by an obstacle, which they were required to remove to access the food. The obstacle would only move in one direction, forwards for some rats and backwards for others. After being trained either to push or to pull in this manner, the rats were confronted with a transfer problem in which the direction was reversed. In the second test of skill, rats were trained to open a door at ground level in order to enter a food compartment. After they had acquired this task, the floor of the apparatus was lowered so that the animals had to climb a ladder to reach the goal box door. Once at the top of the ladder, the rats had to perform a complicated manoeuvre involving holding the ladder rungs with their feet, whilst using teeth and front paws to open the door.

The task of learning to remove the obstacle from the alley was accomplished in the same time for both the EC-IC and EC-SC groups. It was only when reversal was required that EC animals were superior to their IC littermates. Interestingly, no EC-SC reversal differences emerged. Morgan concluded that isolates were not deficient in motor skills per se, but that they have a "reduced capacity for behavioural inhibition" as evidenced by their poor performance in the reversal task. It seems that having learned one way of removing the obstacle, the isolates were slow to change

it. The results of Morgan's second test of motor skill, however, revealed differences between the EC-IC groups, in that the isolates were slower to climb the ladder than their EC counterparts. Time to open the goal box door, however, did not separate the groups significantly.

In a more recent experiment, Henderson (1977) investigated the relative effects of cage size, enrichment and climbing experience on a task requiring extensive motor skills to reach a food hopper, in mice. Results indicated that neither the increased size of the enriched environment, nor the extended climbing practice received in those environments were sufficient to improve performance. Even without climbing experience, mice reared in environments containing a variety of objects showed significantly better later performance than animals reared in comparable but empty cages. Only when the enriched rearing environment was designed in such a way as to severely deprive animals of opportunities for certain motor practice did an appreciable decrement in performance occur, and in this case a relatively short practice period substantially eliminated what appeared to be a simple motor deficit. Henderson concluded that "explanations of enhanced performance resulting from enrichment which are based... on postulated improvements of simple motor skills did not appear sufficient to explain a number of enrichment studies with rodents" (p487). It is clear, however, from his data, that irrespective of possible causes, enrichment produces an animal with superior motor performance.

Probably the most complete examination of EC/IC differences in motor capacity undertaken to date, was that of Lamden (1985). Chosing tasks that did not in themselves involve an extensive learning component and yet which might detect differences in basic motor capacities which are likely to be of importance in a variety of learning situations, Lamden investigated EC/IC differences in muscular strength and maintenance of equilibrium. Reasoning that the reduced motor experience implicit in the impoverished condition would produce rats which are inferior in their motor capacities when compared with their EC counterparts, Lamden employed three tests: the *grip test*, used to assess the strength of forepaw grip when the animal was required to support its own weight; an *elevated runway*, used to measure balancing ability on a narrow track and a

balancing test, designed to give a measure of EC/IC ability to remain in contact with a small surface under stationary and rotating conditions.

Results of these tests showed that EC animals generally proved to be superior to their IC littermates. In particular, EC animals gripped on for longer, when the angle of subtention of the grip test ledge from the vertical was at 45 degrees and at 60 degrees, although in several instances they were observed to lever themselves onto the ledge using their forepaws, thus altering the nature of the task. With respect to the elevated runway, the EC group appeared to manifest superior balancing ability and were less reluctant to attempt to cross the interconnecting track. Once on the track, they took less time to reach the opposite pedestal. Lamden points out, however, that on the narrower tracks employed, the larger size of the IC animal might have represented somewhat of a disadvantage. Furthermore, variations in emotional responsiveness to the test situation may also have contributed to the performance of the groups. However, the contention of EC superiority in balancing was certainly supported by data from the stationary stage of the balancing test. When the pedestal was rotated, contrary to predictions, the IC animals maintained their balance longer. This latter result could have resulted from the heavier IC animal being more stable, or from the greater motor experience of the EC animal, which would make stepping down from the apparatus easier for them to execute, under those conditions. On the whole, however, Lamden's results support her conclusion that "EC rats are superior to IC rats in terms of muscular strength, balance and the precision of their ambulatory movements" (p352).

In summary, of the studies that have investigated the motor skills of differentially housed animals, all demonstrate a degree of EC superiority over SC and IC littermates. However, it is apparent that neither the extra cage size, nor the climbing experience afforded by the enriched environment alone can account for this ability (Henderson 1977). Furthermore, length of exposure to the environment is important. Recently, Cheal, Foley and Kastenbaum (1986) have reported that enriched experience for one hour a month has no effect on motor behaviour as measured by jump down or clinging latencies. Finally, activity, emotionality and weight differences between

the groups may also be implicated in motor abilities. All of these factors need to be examined further, before any firm conclusions can be drawn.

2:3:4 PLAY AND SOCIAL BEHAVIOUR

"An important part of the world of most species of rodents is the realm of social interaction. *Rattus norvegicus* (sic) especially, is highly sociable and it might be expected that some aspects of social interaction could be affected by manipulations of the environment that produce measurable alterations in the nervous system" (Renner and Rosenzweig 1987 p 41).

In spite of the plausability of this hypothesis however, two recent reviews of this area of literature (Dalrymple-Alford, Benton and Brain 1983; Renner and Rosenzweig 1987) have both reported the absence of investigations wherein the social behaviours of EC and IC animals are compared *directly* to each other. Instead, the examination of the social behaviours of differentially housed animals has typically been confined to EC-SC and SC-IC comparisons, with some reference to the voluminous isolation literature (Baenninger 1967).

To further complicate the issue, social behaviour and play have been investigated as both the independant and dependant variable. Emphasis on the former experimental paradigm has resulted from the suggestion that play may mediate the effects of environmental enrichment. In particular, Einon (1980a) believes that "play is not simply practice for later social interactions, but that it affects the versatility of adult behaviour and the ability to learn" (p936). Moreover, Fagen (1981) has written, concerning the cerebral effects of differential environments, that "the specific experience responsible for these changes is participation in playful social interaction, playful object manipulation or performance of playful movements" (p284).

The only study to date which has directly compared the social behaviour of EC and IC animals is a recent publication (Saari et al 1990a) investigating the colony-intruder test in animals exposed to differential environments following neonatal norepinephrine depletion. In this procedure ex-

perimental animals were introduced individually into a well established colony of male Wistar rats for five minutes and social encounters with colony residents recorded. Contrary to their experimental predictions IC animals showed little fighting behaviour, which the authors attributed to "age and weight differences between the colony animals and the expeimental animals or to the strain of rat used in the experiment" (p433). Indeed, the main experimental effect emerged in what the authors called "affiliative" behaviours, EC animals being more likely to engage in social contact than their IC counterparts [10]. In a second experiment in this study two measures of behavioural dominance were examined, water-tube competition [11] and platform-dominance [12]. EC animals were found to drink earlier (in the queue) more frequently and longer than their IC counterparts, as well as dominating the platform, both in terms of mounting order and mounting frequency [13]. Neither of these results is surprising given the fact that EC animals are well used to competing for access to water bottles compared with their IC counterparts as well as climbing and defending platforms from intruders. Indeed, the latter result may also reflect superior EC motor skills rather than social skills per se. However, some clearer evidence about social behaviour in these animals has emerged from EC-SC and SC-IC comparisons, which will be described below.

One of the earliest reports of the effects of differential experience on social behaviour was that of Clarke, Heron, Fetherstonhaugh, Forgays and Hebb (1951) in which terriers were raised either as pets or group housed in a cage 3ft x 6ft, which was specially designed to admit light but to prevent the animals from seeing outside. Peculiarities in the behaviour of the cage reared animals were marked. In particular, although they appeared to be eager for human attention, they strongly avoided handling and displayed either freezing or avoidance behaviour in the presence of the experimenter. Peer interactions were also affected by early experience. In a competitive situation (fighting for a bone) restricted animals were subordinate to all the free environment animals and when placed in a novel environment with another dog, tended to ignore it. These results were

[10] NB: Neonatal depletion of norepinephrine eliminated EC-IC differences in this test of social behaviour.
[11] Where animals compete for access to a drinking tube having been water deprived.
[12] Here order, frequency and time spent on a platform suspended over a swimming pool is recorded.
[13] As before, NE depletion altered the EC-IC effects.

interpreted as evidence of marked disturbance in both the social and motivational behaviour of the restricted animals.

Since this early work, isolation at any age has been found to increase aggression and timidity in most species including man (Heron 1965; deFeudis 1975) and with respect to rodents in particular, isolates have been found to be less skilled in providing aggression-inhibiting cues to other rats (Luciano and Lore 1975). Indeed, the work on isolation and its effects on subsequent behaviour is legendary. For example, Harlow and his colleagues at Wisconsin have shown that monkeys isolated for the first year of life never develop normal social and sexual relationships (Harlow and Harlow 1965) and Einon (1980a) has drawn a parallel when describing the fate of children locked away in cupboards and rooms, deprived of play, stimulation and affection, emerging as "severely intellectually impaired".

With respect to the EC-SC-IC literature per se, far less research has been carried out on the effects of differential environments on play and social behaviour. However, studies have been made of social interactions whilst in the environments. For example, Baenninger (1967) reported that deprived of social contact, isolates engage in more attentive immobility, pawing behaviour and tail manipulation than their group housed counterparts, but that the absence of any social stimuli did not prevent the development of non-social behaviours. For group housed animals, social behaviour varied over time, reaching a peak at 30 to 36 days, decreasing for the next two weeks and remaining at about the same level thereafter. Juraska and Meyer (1986) in an investigation of the patterns of interactions with the social and physical aspects of the enriched environment, found that rats spent considerably more time interacting with objects than in social interactions and that the types of interactions varied little over the length of the study. A few sex differences were noted, such as in play fighting, but these differences were small and not consistent across replications. Indeed, male and female rats were generally found to interact with the environment in a similar manner.

The only *comparative* study to date of the home cage activity of differentially housed animals

is that of Renner and Rosenzweig (1986b) [14]. Defining play so as to only include social play, they videotaped home cage interactions of group housed and enriched animals over a range of behaviours at 45 and 60 days of age. Comparisons of the profiles of social activities in EC and SC cages showed the differences between the groups to be nonsignificant. In a second experiment in the same study, animals' social interactions were measured at 105 and 120 days of age. At 105 days, intergroup differences in social interaction were revealed, the difference primarily being due to an increase in wrestling and chasing in the EC group relative to their SC littermates. In a second set of observations, however, the SC group exceeded the EC in wrestling and the groups were similar in chasing. The later sample, at 120 days did not reveal significant group differences in profiles of social interaction. The nature of these results notwithstanding, the fact that the presence of cagemates makes some contribution to the effects of environmental enrichment cannot be denied. Socially housed rats have shown brain differences from isolates, typically intermediate between EC and IC values (Rosenzweig et al 1978). In such cases the only differences between IC and SC environments are in gross space available (higher in the SC to control for crowding) and the presence of cagemates. The nature of the contribution made by cagemate presence is not clear from the results presented so far, but a possible explanation may be found in the phenomenon of local enhancement and the related concept of social facilitation, described by Thorpe (1963). In local enhancement, the activity of one animal attracts the attention of another, who moves towards the action. This has the effect of directing the newcomer's attention towards whatever the original actor is doing. In cases where the original actor was investigating or interacting with an object, the net effect is further enrichment of the newcomer's stimulus world, as this new attention may lead to independant investigation. This can occur without social interaction as the two animals may never come into physical contact and is reminiscent of Juraska and Meyer's (1986) findings. According to Renner and Rosenzweig (1986b), further studies should involve measurement of local enhancement and investigation of the possibility that there could be more local enhancement of activity in EC than in SC, due to the increased range of activities

[14] This is based on Renner's (1984) doctoral dissertation.

possible in a cage with interesting stimuli to investigate and manipulate. This would redirect the study of the importance of group housing away from social interactions towards gregarious behaviour, or the effects of the mere presence of conspecifics on behaviour. In addition, they suggest (Renner and Rosenzweig 1987) that "although little evidence has emerged to date of any... environmentally induced effects, it remains possible that some specific aspects of social interaction (eg: relative skill in providing clear signals in social communication with conspecifics) are altered by differential environments. In view of the importance of social interaction in the everyday existence of (the rat), further research into possible environmental influences on social interaction is clearly warranted" (p42).

As well as the study of social behaviours of differentially housed animals per se, as mentioned earlier, social behaviour has been investigated as an independant variable. Probably the best known research of this kind was that of Ferchmin, Bennett and Rosenzweig (1975) who were interested in the effects of active interaction or "direct contact" with the environment on *brain changes.* Indeed, in a later report, Ferchmin, Eterovic and Levin (1980) the notion that active interaction with a complex environment is required to elicit brain changes, has been extended to suggest "that environment-dependent brain changes are triggered by play" (p49).

The effects of exposing an animal to social experience on its subsequent *behaviour* has also been the subject of investigation. Arguably, all the papers described in this chapter could be included under this heading, but for the purpose of this present review, only the research that has specifically set out to address this issue will be included. This work can be loosely termed the "play hypothesis" and traces its inception to Einon, Morgan and Kibbler (1978). Earlier work by these authors (Einon and Morgan 1977) had suggested that some of the behavioural effects of keeping rats in impoverished conditions might be caused by *social isolation* prior to 50 days of age. In order to examine this hypothesis more fully, in their 1978 study, Einon et al examined the effects of complete and partial deprivation of social contact between 25 and 45 days of age, on adult behaviour measured in terms of habituation of locomotor activity and object contact in the

open field. They further implicated the importance of social contacts by manipulating the nature of these contacts. In particular, the quality of social interactions was varied, by allowing rats contact with drugged and undrugged companions. Animals totally deprived of social experience (IC) were slower to habituate than animals living in small social groups (SC). Rats allowed one hour of social contact (partial isolates PI), but otherwise living in isolation were intermediate between the IC and SC animals. When the quality of social interactions of the PI was altered by drugging their social partners either with amphetamine or clorpormazine, thus altering the nature of their social contacts, these animals differed from the PI animals in the direction of the IC animals, when measured in the open field. Observation revealed that injection of one of the partners considerably altered social interactions in the pair. The authors concluded that "normal development in the rat may depend upon the flexibility of behaviour encouraged by the early social situation" (p213) and that "the crucial problem for the isolated rat is that it lacks experience in the rapid alternation of roles and behaviour patterns that is characteristic of social interaction in infancy" (p224).

The finding that rats can be protected from the deleterious effects of isolation by short daily periods of social contact, particularily if this contact consists of rough and tumble play, prompted Einon, Humphreys, Chivers, Field and Naylor (1981) to examine species that do not normally play, predicting that these animals, unlike the rat would not show permanant behavioural deficits if isolated prior to 50 days. In their first experiment, short term and long term effects of social isolation on rats and mice were compared. As mice do not play, it was predicted that long term effects of isolation wold be absent in the mouse and that short daily periods of social contact (PI) would have no influence on the behaviour of this species. The results confirmed and extended previous findings. Rats raised in isolation demonstrated permanant deficits in habituation, but partial isolates were indistinguishable from SC animals in open field tests at both 45 and 70 days of age, the latter test following 20 days of SC for all animals. Mice, on the other hand showed a very different behavioural profile. Partially isolated mice resembled isolated mice, suggesting that differences may have been caused by housing mice alone rather than depriving them of all social

contact. This suggestion was reinforced by the finding that social housing of the mice for a period of 20 days eliminated the differences in behaviour of SC-PI-IC mice In a second experiment, rats, guinea pigs and gerbils were given differential experience (SC-PI-IC) from weaning, rats and gerbils being rehoused at 50 days of age into social groups and tested at 70 days in an open field, whilst guinea pigs were rehoused at 60 days and tested at 80 days. For both the gerbils and guinea pigs, as in the mice, behaviour of the partial isolate was like that of the isolate. In the rats however, partial isolates were like the social animals. As Einon et al (1981) state "isolation produces behaviour differences in mice, gerbils, guinea pigs and rats, but only in rats do these behaviour changes survive a period of social housing. Our results are consistent with the suggestion that some of the long-tem effects of early isolation in the laboratory rat are due to deprivation of the opportunity to engage in social play" (p353).

In a third study in the series (Chivers and Einon 1982) the effects of SC, PI and IC on polecat ferrets, a species that is extremely playful, were tested on an object test and an activity test. PI activity was found to be similar to SC activity, but in the object investigation tests, the PI ferrets generally behaved in a manner more similar to ICs than to SCs. This latter finding was unexpected. Chivers and Einon suggest that "the ferrets were actually playing with the objects, not merely investigating them. If object and social play are not entirely independant of one another an animal deprived of social play might be expected to spend more time in object play, given the chance, than a socially reared animal" (p80). Whatever the explanation, it is clear that "the relationship between object investigation and social rearing is more complex in the ferret than it is in the rat" (p75).

To summarise, therefore, there have been a few descriptive studies of the play and social behaviours of animals reared in differential environments, but to date only one comparative study (Renner and Rosenzweig 1986b) in which no differences in social play were observed between EC and SC animals. Social play has also been employed as an independant variable and as such appears to have profound effects on activity and object interaction in species that indulge in

"rough and tumble" play. Why this might be however, is as yet unresolved (Einon et al 1981).

2:3:5 FEEDING AND SLEEPING BEHAVIOURS

a) Feeding Behaviour

The effects of differential environments are not just confined to the brain, but also affect a range of somatic systems (Walsh 1980). Inded, one of the most commonly noted findings has been the differential development of body weights (Bennett, Krech and Rosenzweig 1964; Zolman and Morimoto 1962; 1965; Shelley 1965; Ferchmin, Eterovic and Caputto 1970; Morgan 1973; Morgan, Einon and Morris 1977; Einon, Morgan and Kibbler 1978; Lamden 1985), in favour of the isolated animal. EC and IC weight differences are usually between 8% and 12% (Rosenzweig, Bennett and Diamond 1972a; 1972b; Greenough 1975) with SC animals assuming an intermediate position between IC and EC (Rosenzweig, Bennett and Diamond 1972c). An exception to this general principle is the finding that in long term studies exceeding 100 days, differences tend to disappear (Ferchmin, Eterovic and Caputto 1970). Furthermore, only ad-lib body weight is reported to increase after impoverished rearing (Shelley 1965; Rosenzweig 1971; Hatch et al 1962). Under restricted feeding conditions, the EC-SC-IC differences cease to exist (Morgan 1973).

An obvious question concerns the factors which contribute to this differential development and several contenders including skeletal and adipose systems as well as specific organs have been examined. Informal observations by Fiala, Snow and Greenough (1977) failed to detect differences in skeletal size, but a more precise quantitativie analysis (Diamond, Rosenzweig and Krech 1965) noted significant differences in the dimensions of the skull. As Walsh (1980) points out "it therefore seems quite possible that differences in the remainder of the skeleton might also be detectable by precise measurement" (p85).

With respect to the internal organs, in a series of experiments involving over 400 subjects, consistent differences in liver, spleen, adrenal, testicular, heart and pituitary weights were found

in favour of the isolates, at a variety of different ages (Cummins 1973; Cummins and Walsh 1978). These findings have been corroborated by Geller, Yuwiler and Zolman (1965), and by Ferchmin, Eterovic and Caputto (1970). With respect to adipose tissue, Fiala et al (1977) have noted, as an informal observation, that isolates have more extensive deposits of this type of tissue.

A number of questions concerning these ad-lib weight differences have been addressed in the literature, although according to Lamden (1985) in recent years two main lines of enquiry have emerged.

1. Are the EC-IC body weight differences due to differential food intake, or to some other factor such as differential opportunity for exercise or differential metabolic rates?
2. Whether or not there are differences in gross food intake, are there differences in patterns of food intake?

With regard to the first of these questions, there is evidence of increased food intake in IC animals. The first report of this compared isolates with socially housed groups (Shelley 1965) and found that SC animals ate less food per day, averaging 80% as much food as the singly reared subjects. Furthermore, Shelley found that dropping of pieces of food occurred to a greater degree in the group housed cages. Comparison of EC-IC animals' intake was first noted by Tagney (1973), whilst studying these animals' sleep patterns. She reported, quite incidentally, that IC rats consumed more pieces of food than their EC littermates. However, these data made no correction for food dropping or spillage, an oversight that was rectified by Fiala, Snow and Greenough (1977). Their data indicated that the "widely reported weight differences between EC and IC rats is at least in part a function of higher food intake by the IC's" (p539). Put quite simplyas the title of their paper suggests "IC animals weigh more because they eat more". This conclusion has also been supported by Baenninger (1967) and Lamden (1985). In particular, Baenninger (1967) compared normal laboratory rearing with social isolation during the pre and post weaning period (days 3-92) and reported both eating and drinking to be increased in the

isolated group. Baenninger suggested moreover, that the difference in liquid intake would have been greater were it not for the fact that the group reared subjects had to share the same water nozzle with 5 other rats and therefore stayed at the nozzle longer (the competitive aspect of normal laboratory housing had the effect of increasing water intake, reminiscent of Zajonc's (1965) concept of social facilitation). Lamden (1985) compared EC and IC reared animals in a detailed food monitoring procedure developed by Blundell and Latham (1978). This "microanalysis of feeding" yields a number of different feeding measures, including gross food intake and overall, impoverished animals were found to eat significantly more than their enriched littermates.

On the second question of possible differential patterns of feeding in EC and IC animals, findings are less consistent. Baenninger (1967) noted that isolated animals ate more frequently than group housed controls, but no more details are given in her report. Morgan and Einon (1975) have demonstrated by the measurement of home cage food intake on an ad-lib schedule, that isolated animals ate significantly more than the social group animals during the day, but that the night time difference was negligible. Furthermore, Morinan and Leonard (1980) have also found isolates to eat more during the day than socially housed animals, but not at night. However, Lamden (1985) found the opposite, namely no daytime EC/IC differences but greater IC than EC consumption at night. The reasons for this difference in findings are not immediately apparent, although the use of an SC environment by both Morgan and Einon (1975) and Morinan and Leonard (1980) versus the EC used by Lamden (1985) might have had contributary effects.

An interesting feature of Lamden's work, however, was the detail with which she examined EC-IC feeding patterns, measuring frequency of meals, size of meals and rate of eating. Over a ten-day period, no significant differences were found between the groups with respect to number of meals taken. However, IC animals were found to eat larger meals and to eat these meals more quickly than their EC littermates.

To date, it is still not clear why EC, SC and IC animals should display differential food intake and feeding patterns, although a number of hypotheses have been forwarded. These will be outlined

in the following paragraphs.

It has been suggested that socially housed animals eat less because they are distracted by other animals. Indeed, Shelley (1965) attributes his socially housed animals' food-dropping behaviour to the role of distraction. Conversely, it has been suggested that food intake is increased in IC animals because there is little else to do (Premack and Premack 1963). Indeed, Fiala et al (1977) have noted that isolates may simply eat to relieve the boredom of their situation. Futhermore, they suggest that toys in the EC cage may provide an alternative outlet for some "gnawing" need which exists in rats, observing that the toys in their EC cage were heavily chewed and that survival time for wood and plastic toys was extremely short. Thus IC's might simply be using food to provide for this need. An alternative explanation is based upon Mayer's (1968) contention that an organism must reach some minimal level of exercise before it begins to regulate intake in accordance with need. Mayer noted that food intake is greater in rats at extremely low levels of activity than at moderate levels. Clearly, differential opportunity for and participation in exercise appears to exist between EC and IC environments and must therefore affect animals cage activity levels. However, these are not the only explanations that have been proffered.

Morgan and Einon (1975) have suggested that isolates eat more, as the absence of body heat from other rats requires them to eat more food. Furthermore, Sahakian, Burdess, Luckhurst and Trayhurn (1982) have found that IC rats have a preference for high protein foods such as cheese. Given that intake of energy as protein has been found to have a greater postprandial thermogenic effect than energy as carbohydrate (Zed and James 1980), Sahakian et al (1982) believe IC animals are seeking to increase heat production. Finally, increased IC food intake has been attributed to impaired behavioural inhibition mechanisms (Morgan 1973) in these animals, characterised by their general tendency to persist for longer than controls in whatever they happen to be doing (Morgan, Einon and Nicholas 1975). Feeding behaviour, presumably, would be no exception.

Whatever the explanation, clearly the EC, SC and IC differences in body weight and more

particularily in their patterns of feeding, have implications for their performance in behavioural test situations which make use of a food reward. Indeed, Morgan (1973) has reported that isolates gained more weight than either SC or EC groups on an ad-lib feeding schedule, but that this difference not only disappeared when restricted feeding was introduced, but was actually reversed. This reversal of the body weight difference under deprivation, would suggest that under food motivated learning exercises, IC rats are tested at a lower percentage of their free feeding weight than are either EC or SC groups. This in turn suggests that IC animals may be more motivated than EC or SC animals for food reward. This however, does not necessarily imply enhanced performance in the IC group. As Lamden (1985) points out, "if for example the relationship of motivational level to performance is an inverted 'U', above a certain critical level, increased motivation will lead to a performance decrement" (p112). Whatever the details of differences in response requirements and reward in a given test situation, the possibility of differences in motivation levels between EC and IC groups must be taken into account.

To summarise, therefore, clear differences exist in the feeding behaviours of EC, SC and IC animals. Isolates eat more, have larger meals and eat faster (Lamden 1985). Factors that have been postulated as accounting for this differential intake are many and according to Walsh (1980) it is likely that some combination of these factors will provide the most comprehensive explanation.

b) Sleeping Behaviour

"The relationship between learning and memory processes and the organisation of sleep and waking, including the balance between slow wave sleep (SWS) and rapid eye movement (REM) sleep [15] has long attracted considerable attention among psychobiologists" (Renner and Rosenzweig 1987 p 35). Indeed, the learning opportunities afforded an animal in the enriched condition has prompted investigators to examine the sleep patterns of differentially housed animals. In this

[15] REM is also known as paradoxical sleep (PS) or active sleep (AS)

section, studies that have investigated EC, SC and IC patterns of SWS and REM sleep will be reviewed.

Since increases in REM sleep have been noted in rats after exposure to learning situations (Lucerno 1970; Le Conte and Hennevin 1971; Holdstock and Franks 1971), Tagney (1973) hypothesised that EC rats, presumably having more to learn, might display greater REM sleep than their IC counterparts. Results supported this hypothesis, with EC rats displaying significantly more total sleep than their IC littermates, with this difference emerging in both SWS and REM sleep times. (The percentage of REM sleep in total sleep was thus no greater in the EC animal). After 80 days in the environments, 5 IC rats were transferred to the enriched environment and after 16 days of EC experience, were measured over a 23 hour period. These transferred rats had significantly increased SWS times and REM sleep also increased slightly. Tagney interpreted these results in the light of several kinds of evidence that suggest that sleep is the time in which the brain is in an optimal condition for the synthesis of macromolecules required for its maintenance and restoration (Feinberg, Braun and Shulman 1969; Oswald 1969; Parker, Sassin, Mace, Gotlin and Rossman 1969). In particular, she suggested that her findings of increased EC sleep were due to their increased cerebral activity and consequent requirements for a longer period of the aforementioned optimal conditions for restorative macroprotein synthesis. High levels of physical activity in the EC did not, however, appear to contribute to the increase in sleep, according to Tagney, as Webb and Friedman (1971) have reported no overall alteration in amounts of sleep in rats having long term (70 days) access to activity wheels.

Following Tagney's early work, there have been several studies that have replicated and extended her original findings. In particular, Lambert and Truong-Ngoc (1976b) found that enriched rats slept longer than impoverished animals and that there were significant differences in total REM time and in total SWS time, similar to Tagney's findings. Lambert and Truong-Ngoc also reported increased numbers of REM sleep phases in the EC animals. Furthermore, EC rats were also found to have a higher threshold of activation of cortical responses following reticular formation

stimulation (Lambert and Truong-Ngoc 1976a; 1976b). However, reticular excitability and sleep behaviour were uncorrelated and it was concluded that the environment had independant effects on these two phenomena.

Similar trends have also been reported in kittens. Isolation reared animals slept less than group reared and handled kittens (McGinty 1971) and when isolates were exposed to complexity, the percentages of time spent in both REM and SWS increased. Kiyono, Seo and Shibagaki (1980) also investigated number of REM phases, duration of REM and percentages of REM amd SWS. Unlike Lambert and Truong-Ngoc, these authors found no significant differences in number of REM phases, a finding corroborated both by Kiyono et al (1981) and by Mirmiran, Van Den Dungen and Uylings (1982). However, duration of REM and percentage of REM were elevated in the EC animal during the day time recordings (Kiyono, Seo and Shibagaki 1980) and duration of REM was significantly greater in the EC rat, at night. Interestingly, they report no significant differences between the groups in percentage of SWS, a finding that has been noted by other investigators, using mice (Gutwein and Fishbein 1980a; 1980b).

Differences in sleep patterns and in particular REM parameters between EC and IC animals could reflect an increase due to enrichment or a decrease because of the "frustrating isolation in small cages" (Kiyono et al 1980, p189). In order to investigate this in a subsequent experiment these authors (Kiyono, Seo and Shibagaki 1981) included a control SC group to further investigate the relative contributions of EC and IC to the observed sleep pattern differences between the groups. As with their previous study, percentage SWS and percentage REM were increased in the EC animal when compared to the IC group. However, in this experiment, no differences emerged between the SC and IC groups in the majority of measures taken, suggesting that the now familiar EC/IC sleep patterns are mainly due to enrichment rather than impoverishment.

These differential sleep patterns in EC and IC animals have been studied longitudinally by Mirmiran, Van Den Dungen and Uylings (1982). Using enriched, standard and impoverished rearing conditions, EC animals were found to have a) more quiet sleep time (SWS) b) more active

sleep time (REM) and c) shorter active sleep latency, that is latency to onset of REM sleep. These differences were evident by the third week of environmental conditioning, became statistically significant by four weeks and continued to increase throughout the rest of the enrichment period. None of the sleep parametres showed any significant differences between the SC and IC groups, confirming Kiyono et al's (1980) findings. Indeed, Mirmiran et al's results are in line with the observations of both Tagney (1973) in rats and McGinty (1971) in kittens and extend their findings to include an examination of the enrichment period itself.

Probably the most extensive investigations of sleep patterns, in terms of the number of variables examined, are those of Gutwein and Fishbein (1980a; 1980b). In their first paper, environmental enrichment was found to result in a significant and selective increase in paradoxical sleep (REM). Conversely, impoverished mice exhibited a decrease in REM relative to socially housed controls. In particular, EC mice exhibited a greater number of REM episodes (reminiscent of Lambert and Truong-Ngoc's 1976b findings in rats) a longer average length of REM episode and an increased percentage of REM sleep (percent REM/Total Sleep Time) compared to SC animals. IC mice showed shorter average length REM episodes compared to SC controls and exhibited a considerable reduction in the number of REM episodes, average length of REM episodes and percentage REM time when compared with EC mice. Results were interpreted as providing considerable support for the contention that REM or conditions compatible with REM occurrence are a prerequisite neurobiological process for the maintenance and stability of long term memory. Furthermore, they believe that enriched rearing can attenuate the rate of forgetting.

In their second paper, Gutwein and Fishbein (1980b) examined sleep circadian rhythmicity following enriched and impoverished environmental rearing. Mice were reared in a superenriched environment, as well as the more traditional EC, SC and IC groups. Both enriched groups showed a general increase in total SWS, but the number of SWS episodes, mean duration of SWS episodes and percentage SWS/total sleep time was not significantly different from the SC group for the day cycle. Enriched rearing, however, did produce a selective and significant increase in the

number of REM sleep episodes, mean duration of REM episodes, total amount of REM time and percentage REM/total sleep time throughout the 24 hour cycle. Impoverished mice also showed a general increase in SWS primarily during the day cycle, but exhibited significant reductions on all measures of REM occuring exclusively during the day cycle. The results of this study clearly demonstrate that prolonged rearing in qualitatively different environments alters the amount and quality of sleep and its circadian rhythmicity.

Several explanations of these sleep pattern differences have been postulated. Walsh (1980), for example, believes that the findings of greater EC sleep time and cortical activation threshold (Lambert and Truong-Ngoc 1976a; 1976b) are consistent with the hypothesis that one of the mechanisms mediating the effects of differential environments on brain chemistry and physiology is the arousal response (Walsh and Cummins 1975). That is, that the differential arousal levels engendered by EC, SC and IC rearing would affect their sleep patterns. However, it should be pointed out that experiments involving long-term amphetamine induced hyperarousal in rats have failed to show any developmental effects on regional brain weights (Bennett, Rosenzweig and Wu 1973), a finding that might have been predicted by Walsh and Cummins' (1975) hypothesis. Moreover, Ferchmin and Eterovic (1977) have concluded that arousal per se is not sufficient to induce cerebral changes in EC rats, but that "active interactions" with the environment are a necessary but not sufficient cause for this effect.

Probably the most viable explanation of the differential EC, IC and SC sleep patterns lies in the findings mentioned earlier in this section, that amount of sleep and in particular REM sleep is increased after learning experiences. Indeed, Rosenzweig, Bennett and Diamond (1972) have proposed that learning events underly the morphological differences amongst differentially housed animals. Given this, it seems likely that the reports of increased amounts of sleep throughout the period of EC rearing are reflections of the greater opportunities for learning, afforded by this type of experience. Consistent with this argument, Mirmiran, Uylings and Corner (1983) have found that chronic REM deprivation in early post natal life causes a reduction in the ability of

the cortex to grow in response to sensory (EC) experience later in life.

Whatever the explanation and it must be admitted that the two proferred above are not mutually exclusive, several behavioural facts have emerged from this review. Overall, EC animals have greater total sleep time than their SC and IC littermates (Tagney 1973; Mirmiran et al 1982), longer durations of REM sleep, shorter REM latencies and in some studies a greater percentage of REM sleep (Kiyono et al 1980; Gutwein and Fishbein 1980a; 1980b). Typically, percentage of SWS, number of SWS episodes and duration of these episodes are unaffected by differential experience. Moreover, exposure of previously isolated animals to environmental complexity increases the time spent in both SWS and REM (McGinty 1971; Tagney 1973), whereas chronic REM deprivation has negative effects on the physiology of the cortex.

2:4 OVERVIEW AND CONCLUSIONS

The purpose of this present review was to provide a behavioural profile of animals reared in EC, SC and IC against which to compare the offspring of animals exposed to these differential environments prior to pregnancy, the subjects of this present thesis. Since the early finding that behavioural differences existed between rats kept as pets and those raised in the laboratory (Hebb 1947) there have been numerous studies investigating the behavioural characteristics of differentially housed animals which have provided the focus of this review. Both learned and unlearned behaviours have been studied and from this voluminous and complex literature the beginnings of a clearer profile of EC, SC and IC animals has emerged. In this final section, the key elements of this profile will be highlighted and some conclusions about the nature of enrichment as opposed to impoverishment drawn.

In any attempt to clarify the main findings of a particular literature, a critical evaluation of the quality of the contributing studies needs to be undertaken. When subjected to such a scrutiny, certain criteria need to be established against which to judge the relative merit of any specific experiment. Within the EC, SC and IC literature, as has become apparent in this review, some such criteria have already been suggested (Bennett et al 1970; Rosenzweig 1971a) including a detailed description of what constitutes an enriched or impoverished environment and the number of animals that should realistically be maintained in said environments. In particular, amongst many of the researchers in this field there is now a consensus of opinion which suggests that the nature of the EC, SC or IC should follow the specifications detailed by Rosenzweig, Bennett and their colleagues at Berkeley (Bennett et al 1964; 1970). Furthermore, that the number of animals contained in each type of environment should be more consistent across experiments, with the EC housing 10-12 animals (Bennett et al 1970) the SC 3-5 animals and the IC housing animals individually.

When the four hundred odd experiments reviewed in this chapter are considered, it rapidly becomes apparent that quite a large number of studies do not conform to these simple specifi-

cations. Taking just one of the test situations reviewed, the Hebb-Williams maze, very different types of environmental experience have been employed within this category of studies. For example, Hymovitch (1952) employed a free environment comprising alleys, elevated runways and small enclosed areas and incumbents were compared with animals maintained in enclosed activity wheels and stovepipes. Ravizza and Herschberger (1966) used varying forms of motor restriction as their experimental condition, whereas Aubrecht (1974) compared isolated animals with animals raised in pairs. Admittedly, the latter group can be seen as a form of standard housing, however, this needs to be compared with Brown (1968), who maintained his *twenty five* SC animals in a bare enclosure and compared their performance with his "restricted animals" housed in groups of three.

More damning, however, is a second set of methodological criteria which suggest, amongst other things, that to adequately evaluate the relative contributions of enrichment as opposed to impoverishment to any particular behaviour, a standard housing (SC) condition should always be included as a control environment (Bennett et al 1964). Of the large number of studies reported in this chapter, only 18 studies, that is less than five per cent, have included all three types of environmental housing [16]. The implications here are simple: any firm conclusions drawn should only be based on these few and more methodologically distinguished studies. However, in the author's opinion, under certain conditions these rather stringent criteria can be waived, to allow at least some tentative conclusions to be made about the nature of enrichment and impoverishment. In particular, in the literature, there are some test situations where a large number of studies, many admittedly with varying (and different) flaws, have consistently found similar results. Despite the lack of standardisation of environmental experience or the appropriate control groups in any one study, under these circumstances the weight of evidence does allow some generalisations to be drawn about the nature of the differential environmental experiences. It should be emphasised, however, that these generalisations are necessarily crude and in most instances

[16]These include Lore and Levowitz 1966; Bennett et al 1970; Greenough et al 1972b; Morgan 1973; Gardner et al 1975; Fessler and Beatty 1976; File 1978a; Klippel 1978; Gutwein and Fishbein 1980a; 1980b; Kiyono et al 1981; Shibagaki et al 1981; Nau et al 1981; Seo et al 1982; Rose et al 1985a; 1987; 1988; Renner and Rosenzweig 1986a.

exceptions to the rule can be documented. In the following paragraphs, the main findings to have emerged from this review will be described and the impact of enrichment versus impoverishment on behaviour distinguished. Discussion will centre around Table 2:24 which contains a succinct guide to this extensive literature.

Considering learning tasks first, in general exposure to an enriched environment produces animals with superior performance in mazes, when compared to either their SC or IC counterparts. Furthermore, enrichment has also been found to improve performance in spatial discrimination as well as pattern and brightness discrimination paradigms. However, none of the studies investigating tactile discrimination found any evidence of superior EC performance. Moreover, in discrimination learning no evidence of IC superiority emerged at all, although, SC animals have been found to be superior to EC animals in two studies (Dawson and Hoffman 1958; Crnic 1983).

The reasons for these generally enhanced EC performances, however, are not so clear. They appear to reflect both a greater use of extra-maze cues and an enhanced capacity to process or store information in the enriched animals, although increased exploration, fear or reactivity, coupled with a failure to habituate and a propensity towards repetitious patterns of limited and circumscribed responding in the impoverished animal may also be contributing to the typical EC/IC differences. Alternatively, it may be that these behavioural differences merely reflect differential arousal levels (Edwards et al 1969) or underlying perceptual abilities (Lamden 1985) in the animals. Unfortunately, the variety of methodological procedures in these test situations make any firm conclusions about underlying causes difficult to draw.

Van Woerden (1986) has noted that the relative novelty of cue stimulus affects EC and IC animals differently in the discrimination paradigm, with EC animals appearing to adapt better to novel cues than their IC counterparts. It may well be, therefore, that the enriched environment is producing an animal which is far better able to adapt to novel test situations than either its SC or IC counterparts. However, the age at which the animal is exposed to its environment is an important factor in the development of the experientially-induced behaviour in certain learning

TEST SITUATION	NUMBER OF STUDIES	MAIN FINDINGS
LEARNING		
A: MAZES	90	78% of papers reviewed have found evidence of superior maze performance in animals exposed to varying degrees of environmental complexity.
1. Hebb Williams	48	Vast majority of studies demonstrate superior performance of animals raised in complex environments when compared with animals raised in isolation or socially housed conditions. The greater the social and perceptual experience, the better the performance. Behavioural effects of isolation can be reversed. Preferable to expose animals to enrichment during adolescence, but enrichment produces positive effects at any age. Only minimal amount of enrichment required.
2. Lashley III	16	Majority of studies have found EC animals to be superior performers. No evidence of IC superiority in this apparatus. Age of exposure to differential environments does not influence performance.
3. Other Mazes	26	Variety of mazes employed (10). Results less consistent than previous maze literatures, reflecting range of methodologies used. 17/26 studies demonstrate EC superiority. No evidence of IC superiority.
B: DISCRIMINATION	27	37% of studies report evidence of EC superiority, but depends on type of discrimination. Greatest improvement in spatial, then pattern, then brightness. No effect in tactile discrimination.
1. Brightness	16	Studies have either found no differences between groups, or evidence of EC superiority (27%). No evidence of IC superiority in this task.
2. Pattern	5	Some evidence of EC superiority (40%). However, too few studies to draw firm conclusions.
3. Spatial	3	Some evidence of EC superiority, too few studies to make firm conlusions, however. Results interact with age of onset of experience and number of hours spent in environment.
4. Tactile	3	No significant differences found between EC, SC and IC animals. All studies involved surgery, so no firm conclusions can be drawn.
C: REVERSAL	23	Differences in reversal learning due to the deleterious effects of impoverishment. Experience should begin imediately after weaning.
D: AVOIDANCE	28	A third of the studies have found evidence of EC superiority, However, EC/IC animals respond differently to exteroceptive stimuli, so care must be taken when drawing any conclusions in this task.
1. Active	12	From these studies no clear pattern of effects has emerged. Some evidence that behaviour is determined by strain of animal and task employed. Only task where evidence of IC superiority emerged.
2. Passive	16	Social and/or perceptual enrichment appears to be efficacious. No evidence of IC superiority.
E: SKINNER BOX	19	Clearer results in simple procedures than are found in the more complex tasks.
1. Simple Procedures	14	Generally, isolates bar press more.
2. Complex Procedures	5	IC animals deficient in the elimination of maladaptive responses. Overall, picture less clear cut and task specific. In DRL experiments, IC animals hampered, in GO-NO-GO, no obvious effects of nature of early experience.

Table 2:24 Summary table of main findings of the studies and/or experiments reviewed in this chapter.

TEST SITUATION	NUMBER OF STUDIES	MAIN FINDINGS
NON-LEARNING		
A: ACTIVITY	167	Although isolates appear to be the most active over a period of time, activity is a complicated phenomenon and interacts with the complexity of the test situation relative to home cage environment.
1. Basal	3	No experiment to date has adequately explored EC/SC/IC basal activity.
2. Reactivity	164	Complex phenomenon (see above).
a. Open Field	52	Variations in open field apparatus, subjects, analysis and interpretation makes firm conclusions difficult. However, activity over trials does distinguish EC/IC profiles. Typically, IC animals maintian higher levels of responding when compared with SC/EC. With respect to rearing/defecation, few consistencies in results.
b. Mazes	25	If significant differences emerge EC animals are less emotional than their group housed or restricted counterparts. With respect to activity/exploration, results diverse, reflecting wide range of procedures.
c. Activity Wheel	25	Higher levels of spontaneous EC activity in activity wheel, but lower levels of EC reactive activity.
d. Novelty	32	Environment: Restricted rats show an enhanced preference for novelty but complexity of test situation must be taken into account. Objects: Object contact time and manipulation demonstrate differences between EC, SC and IC animals. EC animals show greater diversity and seem more inquisitive, with a large repetoire of exploratory behaviours. SC animals are more purposeful in style and more receptive to extra field cues. IC animals make less contacts initially, but over time have higher frequencies of (shorter) bouts of object contact.
e. Emergence	30	Generally, isolates are slower to emerge. This effect can be reversed by social housing.
B: PERCEPTION	7	Conclusions difficult to draw, because of small number of studies.
1. Depth	3	Some evidence of more highly developed depth perception in EC animals. Too few studies to draw firm conclusions.
2. Noxious	4	Social and sensory restriction detrimentally affects responses to low intensity painful stimuli.
C: MOTOR	4	Studies all demonstrate superior EC motor skills. No evidence that this is due to enrichment as opposed to impoverishment. (NB small number of studies)
D: PLAY/SOCIAL	10	Paucity of investigations comparing EC/IC directly, so few conclusions can be drawn. Some evidence that short period of social contact can protect animal from deleterious effects of isolation.
E: FEEDING	10	Isolates eat more than socially housed animals. Differences in patterns of feeding are less obvious, but IC animals tend to have larger and faster meals.
F: SLEEPING	9	EC animals sleep more and display more REM than isolates. Both EC and IC contributes to this effect.

Table 2:24 continued

situations. For example, both Krech et al (1962) and Bennett et al (1970) maintain that enriched experience only results in superior reversal discrimination performance when environmental experience is given immediately after weaning. Interestingly, in reversal learning tasks it appears that performance is impaired by isolation rather than enhanced by enrichment, which Morgan (1973) has attributed to the IC animal's reduced capacity to inhibit a previously successful strategy.

Active avoidance is one of the few learning situations in which the IC animal's performance has been found to be superior to that of its EC peer. To explain this finding Joseph and Gallagher (1980) have accredited the restricted animal with "the selective directing of responses to adaptive ends" (p541). With passive avoidance, however, the opposite is true, none of the studies demonstrating an IC superiority in performance when compared with EC animals. Furthermore, in this paradigm socially housed animals are as successful as their enriched counterparts, a finding which is not surprising given that both of these groups of animals have had opportunities to practice passive avoidance whilst growing up and living with other animals (Gardner et al 1975).

Why passive and active avoidance should produce such different results with respect to relative EC/IC performances is not entirely clear. It may be that the opportunities afforded the socially enriched animals are more transferable to the passive avoidance task than the active avoidance situation. Alternatively, as Renner and Rosenzweig (1987) have suggested footshock of " a particular intensity may be perceived as differently aversive by the two groups". Their "subsequent performance could not then be clearly ascribed to differences in information processing or behavioural abilities" (p47).

More generally, the relative value of reinforcers has been the subject of study in operant conditioning paradigms (Rose, Love and Dell 1986) and it has been noted that brief presentation of light in a bar-press contingent light reinforcement becomes aversive at lower intensities for EC than for IC rats. Einon and Morgan (1978) have argued that IC animals appear to have a general disturbance of inhibitory mechanisms. It may be, therefore, that enriched animals

are responding more appropriately to the more intense stimulation, perhaps even having a more "sensitive" nervous system.

Indeed when the operant conditioning experiments are included, this explanation starts to have some credence. IC animals typically bar press more than their EC counterparts in the acquisition phase. This, it has been suggested might reflect higher activity levels in the IC animals (Coburn and Tarte 1976) or that their behaviour is directed towards maximising sensory stimulation (Lamden and Rose 1979; Rose, Dell and Love 1987). This work suggests that the behaviour of EC, SC and IC animals reflects different levels of sensitivity to stimuli, such that IC animals will tolerate or even seek levels of stimulation that their socially enriched counterparts would find aversive. When considering IC animals' bar press extinction rates, which are slower than their enriched conspecifics, this pattern of stimulus seeking, coupled with a failure to habituate to stimuli (Joseph and Gallagher 1980) begins to make some sense of the data.

So, with respect to learning behaviours, certain elements seem important. Firstly, the early experience of the animals may well interact with the complexity of the test situation to produce the qualitatively different patterns of behaviour observed in the EC, SC and IC groups. Furthermore, both social enrichment and social and perceptual enrichment appear to afford an animal certain opportunities for learning resulting in "skills" which, dependant on the nature of the learning task, can be transferable. Impoverishment on the other hand, produces an animal which according to Einon et al (1975) has an altered developmental process, which may affect its sensitivity to stimulation.

With respect to the non-learning behaviours and in particular general activity, typically, IC animals are more active than either their SC or EC counterparts, but this heightened activity is only really apparent if the animals are tested over a period of time. Furthermore, as with the learning tasks, groups' performances are affected by the nature of the test environment.

For example, although IC animals maintain higher levels of activity in the open field and demonstrate a perseverative tendency to explore the Hebb-Williams maze (Joseph and Gallagher 1980),

their EC and SC counterparts, although initially reactive, tend to reduce their activity over days. This is not surprising when one considers that the open field (and to an extent the Hebb-Wiliams maze) is typically less enriching for EC and SC animals than their home environments (having no toys or conspecifics to interact with). When the novel environment does contain toys, however, EC animals react in an entirely "sensible" manner. Obviously the introduction of a new object is, in itself, not a novel procedure to these animals (after all most forms of enrichment involve rotating a variety of toys into the EC over the enriching period) but their reactions to these objects do contain a degree of caution. Approach is systematic (almost planned) compared to their IC counterparts and interactions are more varied and imaginative. Anecdotally, it seem that the IC animal, already aroused by the novelty of the environment goes into "over-drive" dashing around, jumping with fright and bumping into the objects. Once the initial novelty has worn off, however, the IC animals engage in frequent bouts of repetitive behaviour, as if they were either stimulus seeking, failing to inhibit behaviour or just plain hyperactive.

Transferable skills also characterise the EC, SC and IC groups' perceptual abilities. EC animals have been found to make greater use of extra-field cues (McCall et al 1969), which is not unusual given their rearing experience. In addition, both Eichengreen et al (1966) and Lamden (1985)'s findings in the visual cliff suggest that EC animals are more inclined to use depth cues than their IC counterparts. With the perception of noxious stimuli, the evidence suggests that for the low intensities of shock stimulation IC animals are less sensitive than their EC counterparts. Whether this reflects their need for stimulus, or that for lower rates they are less sensitised than their EC peers is not known. More pragmatically, Lamden (1985) has speculated that the IC level of response reflects the fact that these animals are heavier than their EC controls and may not feel the lower intensities of shock as much.

With respect to motor abilites, as would be predicted, EC animals demonstrate a degree of superiority over the SC and IC counterparts. However, neither the extra-cage size, nor climbing experience afforded by the enriched environment alone can account for this ability (Henderson

1977). It may be that activity, emotionality and weight differences between the groups contribute more to these findings (Lamden 1985), a matter still open to further investigation. What is interesting about these differences, however, is they may aid the EC animal's performance in certain of the learning and activity tasks detailed above, a fact not often taken into account by researchers.

Moving on now to social behaviour, when compared with IC animals EC rats have been found to be more likely to engage in social contact with a "new" colony of rats. This isn't surprising, given that their early experience involves engaging in social contact. With IC dogs (Clarke et al 1951) peer interactions have been found to be negatively affected by their early experience and in general isolation can be seen as inducing timidity and inappropriate social responding (Heron 1965; DeFeudis 1975). Indeed, social behaviour, or more specifically play behaviour has been suggested as one of the important components in the mediation of the enrichment effect (Einon 1980). Whether the EC/IC differences in behaviour reflect the fact that the EC animal is ameliorated by its being allowed to play or that the IC animal is reduced in capacity and follows an abnormal developmental pattern because of its being deprived of play (or both) is still to be resolved. Exposure of IC animals to opportunities for play, however, can ameliorate their early deficits (Einon and Morgan 1977; Klippel 1978).

The final behaviours to be discussed, are those "maintenance" behaviours, feeding and sleeping. Differences have been reported in the feeding behaviours of EC, SC and IC animals, with the latter animals eating more, having larger meals and eating faster (Lamden 1985). It may be that the socially housed animals eat less because they are distracted by other animals, or that the IC animals eat to relieve the boredom of their situation. Alternatively, Morgan and Einon (1975) have suggested that isolates eat more to maintain body heat in the absence of other animals. Furthermore, following the notion that IC animals fail to inhibit behaviours once they have engaged in them, it may be that once having started to eat, these animals can't stop. The importance of differences in feeding patterns between the groups, other than to extend

the behavioural profile of these animals, lies in the implications these patterns may have for the groups' performances in behavioural tasks which make use of food rewards. As part of the increasingly complex pattern of differences between the groups, it now appears that their different performances might also reflect their basic drives for food.

Finally, sleeping behaviour has also been reviewed in this chapter. The rationale for examining the sleeping patterns of differentially housed animals lies in the relationship between the organisation of learning and memory processes and the organisation of sleep/wake cycles. Overall EC animals have a greater total sleep time than either their SC or IC littermates, longer durations of REM sleep, shorter REM latencies and a greater percentage of REM sleep. Slow wave sleep, is apparently unaffected. These results are typically interpreted as resulting from the greater need of the EC animal to consolidate learning acquired in its environment. If, as has been argued in this review, the differential environments afford their inhabitants qualitatively different skills and if, as has also been argued, the EC provides more opportunities for learning than either of the other two environmental conditions, then these sleep patterns are not unexpected at all.

So as can be seen from the above, exposing animals to differential environments can have profound effects on their behaviour in a variety of experimental tasks. The rather complex profiles that have emerged suggest that, as would be expected, qualitatively different animals are being produced by the EC, SC and IC environments. It is extremely difficult, however, to arrive at a shorthand which adequately describes the complexity of the enriched, impoverished and standard housed rats, since there are numerous interactions between the strain and sex of the animal, its environment and the test situation. However, if this is acknowledged, the distinguishing influences of enrichment when compared with impoverishment and standard housing can be summarised as follows:

PRINCIPLE EFFECTS OF ENRICHMENT

- Enrichment produces enhanced problem solving abilities in a variety of test situations.
- These improved skills and strategies coupled with a wide repetoire of exploratory behaviours

and ease of adaption confer on the EC animal various functional advantages including those aiding survival.

- EC animals demonstrate superior motor skills, but these may reflect differences in weight, emotionality and activity between EC and IC animals rather than EC enhanced motor control per se.

- EC animals are more likely to engage in social contact and are more socially dominant than IC animals.

- Enrichment increases the need for sleep and REM activity.

PRINCIPLE EFFECTS OF STANDARD HOUSING

- When compared with enriched animals (and in some circumstances impoverished animals) socially housed animals often appear to be more emotional.

PRINCIPLE EFFECTS OF IMPOVERISHMENT

- Physical stimulation holds different significance for EC and IC animals. For example, isolation seem to induce inappropriate perception of noxious stimuli, which may in part be determined by their inappropriate emotional reactivity in novel environments. However, more generally these animals seem to be motivated towards increasing contingent sensory input and maximising sensory stimulation.

- IC animals have reduced capacity for response inhibition in general and in particular seem deficient in their ability to suppress an overlearned and previously rewarded pattern of behaviour. In addition, impoverishment results in maladaprive development of exploration and play sequences which interact with their poor inhibition of inappropriate responses and may well have a lowered survival ability.

- IC animals are generally more reactive, especially over trials. EC animals, in comparison show higher levels of spontaneous activity but lower levels of reactive activity.
- Isolates appear unable to handle the discrepancy between the limited range of their early experiences and the typical variety and complexity of test situations.
- There is some evidence that isolates have slower decision making times.
- Isolates eat more, have larger meals and eat faster than their socially or perceptually enriched counterparts. They may also be more motivated by food than their SC and EC conspecifics, but this does not necessarily lead to enhanced performance. In addition, isolation reduces the need for sleep and REM activity.

To round all this up and describe the profiles more generally, then, the enriched environment seems to develop in its inhabitants a greater adaptability across a wide range of test situations. Enriched animals have enhanced physiological activity, superior problem solving ability and more directed interactions with their environment including their social environment, where they show greater dominance. Socially housed animals, as would be expected, can translate some of their socially acquired experiences to new situations. However, these animals are less well adapted to novelty than their EC counterparts and can be more emotional. In contrast impoverished animals are generally more reactive, although their activity lacks direction. In addition, their experiences afford them little advantage in their interactions with either their social or physical environments, in some circumstances even producing maladpative behaviours. On a more positive note, however, these apparent deficits can be ameliorated by therapeutic environments, with very short periods of social contact, for example, quickly reducing the deleterious effects of isolation.

CHAPTER THREE: OVERVIEW OF THE LITERATURE INVESTIGATING THE EFFECTS OF MANIPULATION OF THE MATERNAL GENERATION ON OFFSPRING PHYSIOLOGY AND BEHAVIOUR

" And surely we are all out of the computation of our age and every man is some months elder than he bethinks him; for we live, move, have a being, and are subject to the actions of the elements and the malice of diseases, in that other World, the truest Microcosm, the Womb of our Mother."

(Sir Thomas Browne's Religico Medici, 1642)

3:1 INTRODUCTION

As outlined in chapter one, the purpose of this thesis is to examine the effects of differential maternal environments prior to pregnancy on the behaviour of future offspring. Whilst employing EC, IC and SC as a maternal manipulation is rare in the literature, manipulation of the mother as a paradigm for exploring the development and later behaviour of an organism is well documented and provides the focus of this review. Furthermore, as with most research on the effects of early experience, both the *timimg* and the *nature* of the maternal manipulation have been varied and are taken into consideration in this present chapter.

Considering first the *timing* of the experience, typically in the literature manipulation of the mother has occured during pregnancy. However, as Joffe (1982) has noted "not only is the mammalian organism susceptible to the effects of an astonishing range of physical, chemical and biological agents during the period from conception to birth, but for a complete understanding of the outcome, events occuring prior to pregnancy and during the birth process itself have to be taken into account" (p123). This is reflected in the literature where attention has also been paid to the effects of events prior to conception. This last line of research is particularily interesting as it removes the possibility of direct effects on the foetus since no treatment is applied to the mother following conception. Any offspring effects must therefore be mediated by the *maternal* response to the treatment. Additionally, maternal influence can occur during the perinatal period, that is during the time from birth to weaning when the young animal is being suckled by its mother. This is a time when the neonate is particularily sensitive to "psychological effects" (Broadhurst 1961). In the present review, findings from all of these procedures will be examined and for

the sake of simplicity will be presented in chronological order, such that manipulations occuring prior to pregnancy will be presented before those manipulations imposed on a pregnant animal (prenatal procedures) are reviewed, which in turn will precede those maternal influences that occur postpartum.

With respect to the second element described above, the *nature* of the maternal manipulation, perusal of the literature reveals that a wide range of agents applied to the mother can influence the ontogeny of her offspring. However, it is impossible to provide an exhaustive list of environmental agents that affect development. In the first place, if the effects are mild the outcome may not even be recognised as anomalous (Joffe 1982). Secondly, failure to recognise until recently that an agent may affect development, even when encountered prior to conception either by the mother or the father, has resulted in a paucity of evidence on the effects of agents prior to conception and on paternal drug effects (Goldman 1980). Thirdly, even in the case of clear structural or behavioural malformations it is often difficult to establish a causal relationship between an agent and an outcome. Identical outcomes may sometimes result from either genetic or environmental factors and particular agents can produce a variety of outcomes (Barnes 1968), probably as a result of variations in time and duration of exposure and of dosage, or as a result of individual differences in susceptibility (Joffe 1982). Fourthly, the absence of a distinctive effect or patterns of effects means that a possible teratogenic agent is unlikely to be recognised (Wilson 1977a). Furthermore, difficulties in establishing that an agent affects development are exacerbated in the case of functional alterations and delayed effects, that is those not manifested or ascertained at birth. As Joffe (1982) points out, "Not only does delay in the manifestations of the condition mean that a prenatal event is less likely to be suspected, but, in the case of functional alterations, postnatal events can produce effects identical to both genetic factors and prenatal events' (p124).

Despite these problems, Joffe (1982) has outlined what he sees as the main environmental agents which may influence development [1], which are summarised in Table 3:1.

[1] More details, including summaries of probable effects and references to supporting data can be found in Brent (1976; 1977), Catz and Yaffe (1976), Goldman (1980), Grabowski (1977), Wilson (1977a; 1977b) and Winick (1976).

Radiation
Drugs and Hormones
Maternal Metabolic Disorders
Pregnancy/Delivery Complications
Chemicals
Infections
Nutrition
Maternal Stress
Intrauterine Physical Factors

Table 3:1 Environmental Causes of Developmental Effects (Taken from Joffe 1982)

As can be seen from this table, there is a wide range of maternal manipulations that have been found to have an impact on offspring. Whilst some have some relevance to the type of environmental manipulation this thesis proposes to examine, other manipulations, of a more pathological or toxic nature which impose a degree of harm, clearly have not. To be more specific, environmental agents such as radiation, drugs and hormones, chemicals, infections and levels of nutrition although of primary interest to the medical and pharmacological professions, are not directly relevant to the present research. Indeed, of the nine agents listed in Table 3:1, only one *maternal stress* includes procedures which are in any way comparable with the paradigm employed in this present research. Consequently only those procedures which fall within this category will be reviewed in this chapter, the details of which are presented in the following sections, defined by the timing of the maternal manipulation.

3:2 PRIOR TO CONCEPTION INFLUENCES

In the past few years there has been an increased awareness of the effects of influences prior to conception on the healthy development of children. Already, agents such as radiation and possibly some hormones and drugs have been found to cause chromosome damage in the sperm or ova and thus act as determinants of embryonic death or development defects well before conception (Joffe 1982) [2]. Consequently, some proportion of chromosomal disorders can be attributed to

[2] In the case of the damaged ova, such events could exert their influence decades in advance of fertilisation, since the development of the human ova begins whilst the mother herself is a foetus. In the case of the sperm, the

environmental events long before the conception of the affected embryo (Joffe 1982).

Of more interest to the present work, however, are the agents acting prior to conception that can affect development without apparently causing chromosomal damage (Joffe 1982), including those studies which have emerged from two areas of literature in particular, namely the prenatal stress literature (Joffe 1969b; Archer and Blackman 1971; Joffe 1978) and Denenberg's work on programming life histories (Denenberg 1969a). These will provide the focus of this particular section.

One of the earliest investigations of the effects of maternal stress prior to conception on offspring behaviour emerged from a series of studies introduced by Thompson in 1957. His work employed a methodology that consisted of training animals before mating to avoid shock on the presentation of a conditioned stimulus (CS). Conditioned females were then mated and returned to the apparatus during pregnancy, at which time the CS was presented without the shock. This procedure, he argued, subjected pregnant animals to stress, but reduced the possibility of direct effects on the foetus. In the open field, offspring of prenatally stressed females were found to be significantly less active than offspring of control animals, and in an alley test, were significantly slower to run through the alley. Thompson (1957a) interpreted the effects on the behaviour of the offspring as probably indicative of increased emotionality and tentatively attributed this to endocrinal changes in the mothers resulting from the experimental procedure being transmitted to the foetuses. Kaplan (1957) immediately pointed out the possibility that the effects on the offspring may have been caused by maternal hormonal changes resulting from the stress of the training period itself and suggested that the control for such effects was to subject a group to the same training and premating stress as the experimental animals, but not to stress them further during pregnancy. Thompson (1957b) accepted this possibility and added that "radically altering the mother before pregnancy may be equivalent to radically altering the environment during pregnancy" (p74), since this stress during the premating period might produce in the mothers a

susceptible period is up to about 64 days prior to fertilisation, the time required for the maturation of a sperm cell (Goldman 1980).

much lower "threshold of reactivity" to the various normal environmental stresses. This question was submitted to experimental examination by Thompson, Watson and Charlesworth in 1962.

In their experiment, a group of ten female Sprague-Dawley rats were subjected to three shock sessions per day for ten days prior to mating. Each shock session consisted of twelve shocks, administered over a two-minute period. Ten females in the control group were left untreated. After mating both experimental and control females were left undisturbed. Half the offspring in each group were cross fostered and half remained with their natural mothers. When they were between 60 and 80 days of age 32 experimental and 32 control offspring were tested in an open field in which defecation, ambulation and the latency of the animals' activity were recorded on three daily ten-minute trials. Additionally, the animals' speed of running to a food reward in an alley test, after 24 hours of food deprivation was also recorded. No significant overall differences were found between the behaviour of the offspring of females shocked prior to mating and that of offspring of untreated control animals on either test. However the analysis of open field activity showed a significant sex by fostering by prenatal treatment interaction, which although complex, has been described by Joffe (1969b) as follows: "it appears that among females, offspring of experimental mothers were more active than controls, whereas in the case of males, while cross fostered experimental offspring were more active than cross fostered controls, non fostered experimental offspring were less active than non cross fostered controls" (p138). It seems a little surprising then, that Thompson et al (1962) concluded that "stress given prior to conception either by itself or in combination with other variables has little effect on offspring behaviour" (p10-11). As Joffe (1969b) points out, "although the effect of the stress was detected only in a complex interaction, the stress did significantly affect offspring behaviour" (p138).

Following Thompson et al's (1962) work, a second experiment (Ader and Belfer 1962b) in which offspring of female Long Evans rats exposed to avoidance conditioning prior to mating were compared with offspring of animals exposed to both premating avoidance conditioning and prenatal stress, did, however, report differences between the groups. Offspring of gestationally stressed

mothers were significantly less active in an open field at 30 to 40 days, than those of mothers stressed prior to mating. The lack of untreated controls, however, makes it difficult to tell if these results are due to premating stress on its own. In addition, the open field test comprised a single, one-minute trial, a procedure quite likely to give invalid and unreliable results (see chapter two). Furthermore, maternal behaviour was rated daily during the first postpartum week in this experiment, during which a retrieval test was employed, which in disturbing the litter, may well have had confounding effects.

In an experiment in which most of these criticisms were taken into account, Joffe (1965b) found that premating stress did contribute to offspring behaviour. He used three groups of rats; two groups received daily avoidance training for a period of 14 days prior to mating, after which one group continued to receive the CS prenatally. The second experimental group was left undisturbed after mating and the third group, the control group, left undisturbed throughout the whole pre and postmating periods. Offspring from the three groups were tested in an open field and on an avoidance conditioning task at approximately 100 days. Results of ambulation in the open field revealed a significant difference between offspring of the premating conditioning group who were not treated after mating and the other two groups. With the second test, however, a reversal occured, offspring of the premating/gestationally stressed group differing significantly from the other two groups. In his discussion of these findings Joffe (1969b) speculates on some possible routes of these effects and suggests that "changes in offspring behaviour result from neuroendocrinal changes in the mother, transmitted transplacentally to the offspring. The occurrence of effects as a result of procedures terminating before the females were mated supports the possibility first suggested by Kaplan (1957) that effects which are apparently the result of events occuring during gestation may result instead from hormonal changes during the premating training persisting after cessation of treatment"(p222-223). He further points out that "the occurence of effects on offspring as a result of procedures terminating before mating also excludes the possibility that the stress procedure directly affected the foetuses and conclusively establishes the intermediary role of the mother" (p223), a fact which has direct relevance to the present thesis.

This "intermediary role" has been further substantiated by reports in the literature of postnatal maternal effects. Of particular interest to the present section, is a report of postnatal effects of a manipulation imposed prior to conception reported by Ressler (1966) in a personal communication to Joffe (1969b, p20). He found that mice reared by foster mothers given avoidance training for 15 days, 50 trials per day, prior to mating performed better at 50 days of age on an avoidance conditioning task than mice reared by undisturbed foster mothers. There is also evidence from several prenatal stress experiments demonstrating an interaction of prenatal and postnatal variables (Hockman 1961; Thompson, Watson and Charlesworth 1962; Ader and Plaut 1968; Masterpasqua, Chapman and Lore 1976); these will be mentioned in more detail in the following section (3:3).

As well as using avoidance conditioning as a premating paradigm and examining the effects on offspring, one study (Wehmer, Porter and Scales 1970) has extended this work across two generations. In particular, these authors found that grandpups of female albino rats which had been subjected to avoidance conditioning before mating were more active in an open field than descendants of non-disturbed controls grandmothers [3].

Other than avoidance conditioning, two other premating procedures have been reported in the prenatal stress literature, one employing shock traumatisation (Denenberg, Ottinger and Stephens 1962; Gauron 1966; Pereira, Ardila and Figueroa 1980), the other immobilisation (Lane and Hyde 1973). In the former procedure, shock has been applied both in the mother's infancy and just prior to conception. Denenberg, Ottinger and Stephens (1962) found that "the variable of shocking the mother (in infancy) resulted in significant emotionality on the part of the offspring in adulthood and reduced body weight after avoidance training" (p71). In Gauron's (1966) study, in which Sprague-Dawley rats were exposed to three minutes of inescapable shock daily from Day 10 to 25 of life, and their offspring tested in the open field, none of the main effects was significant. However, a significant maternal treatment by cross fostering interaction

[3] This work extends the pioneering study of Denenberg and Rosenberg (1967), the first researchers to demonstrate non-genetic transfer of information across generations, which will be described in more detail below.

emerged, reminiscent of Thompson et al's (1962) findings. Gauron suggested that his findings "led to the conclusion that different combinations of mothers resulted in differential behaviour in offspring. In other words, implications regarding offspring behaviour must take into consideration both constitutional (including physiological and biochemical elements) and environmental factors and the interaction of the two" (p223). More recently, Pereira, Ardila and Figueroa (1980) have reported significant differences between offspring of Swiss albino mice shocked eight days prior to pregnancy and offspring of control animals. Interestingly, in this experiment, no significant differences were found between offspring of mothers shocked prior to pregnancy and offspring of animals shocked during pregnancy, suggesting that this manipulation prior to pregnancy is as efficient a stressor as one imposed prenatally. This finding casts some doubt on Joffe's (1978) cautionary comments concerning the efficacy of preconception stressors.

Lane and Hyde's (1973) study was designed to investigate the effect of maternal stress on both fertility and sex ratio, following reports in the human literature that schizophrenic women produce significantly more daughters than sons. Because their study employed a premating stressor, its findings are relevant to the present review. In particular, stress consisted of placing rats in individual wire screen cocoons which severely restricted motion. Whilst three control females remained in their cages, three experimental animals were stressed for four hours a day for seven days. After the treatment, females were mated and consistent differences were found between stressed and unstressed mothers in both number and sex ratio of their offspring [4], very much suggesting a hormonal mediation.

All of the above procedures have employed "punitive" (McKim and Thompson 1975) stressors. There is, however, one other form of external manipulation of the mother which according to McKim and Thompson (1975) is minimal in nature and which has also been employed as a premating procedure. This manipulation is handling and has been used in conjunction with environmental enrichment by Denenberg and his colleagues in their "programming of life histories"

[4] In view of this finding, in the present thesis, number and sex ratios of all litters were recorded, to see whether differential environments produce differential stress levels.

research (Denenberg 1969a; 1970). Like Denenberg et al (1962) and Gauron's (1966) work where shock traumatisation occured during the mothers' infancy, handling is a perinatal procedure and consists of removing complete litters from the home cage, placing the pups on shavings in a can for three minutes and then returning the pups to their mothers. Handling rats in their infancy has marked effects on their subsequent behavioural and physiological processes (Salama and Hunt 1964; Schaeffer 1968; Denenberg 1968; 1969a; 1977; Levine 1969a; 1969b; Russell 1971; Daly 1973; Lee and Williams 1974; Wong and Wong 1978), in particular causing them to be sexually precocious, to weigh more in adulthood, to be less emotional, to explore novel and social objects more, generally to learn better when noxious stimuli are used as reinforcers and to have a lesser adrenal corticosterone response when exposed to novel stimuli, but a greater response when exposed to distinctly noxious stimuli, than their unhandled counterparts (Denenberg 1969a).

In 1963 Denenberg and Whimbey investigated whether or not these changes affected handled rats' offspring. Reasoning that "modifications of the offspring's characteristics could occur during their fetal period, as a result of physiological changes induced in the mother by the handling she had received in infancy, or they could occur after birth as a result of either physiological changes (which could for example modify milk supply) or behavioural changes induced in the mother by the handling she had received in infancy" (p1192) Purdue-Wistar rats were either handled or left undisturbed in infancy. Some of their offspring were left with their natural mothers, others fostered to mothers of the same experiential background, whilst a third group were fostered to the treatment opposite to that experienced by their biological dam. Starting at 50 days of age, offspring were given four days of open field testing, ambulation and defecation scores over a three minute period being recorded. Additionally, body weights were measured. Results indicated that body weight reflected maternal postnatal experiences; young raised by handled mothers weighed more than pups raised by non-handled mothers, irrespective of natural mother. However, with respect to activity, both the natural mother and the fostermother influenced offspring performance. Young born of non-handled mothers and fostered to handled mothers were more active than the other groups, the next most active group being the complement of

this, rats born of handled mothers and reared by non-handled mothers. These results, according to Denenberg and Whimbey "clearly establish that the experiences which the mother received while an infant were profound enough to modify her offsprings' body weight at weaning and open field behaviour in adulthood" (p1193). Furthermore, they suggested that "these modifications were mediated through both the prenatal mother-fetus relationship and the postnatal mother-young interaction" (p1193). Interestingly, although offspring of non-handled mothers were more active in the open field than offspring of handled mothers, a finding replicated by Denenberg and Whimbey (1968) and Denenberg and Rosenberg (1968), when these animals were given additional experience in adulthood, namely that of getting pregnant and bearing and rearing a litter, effects were exactly opposite to earlier findings. In this instance, offspring born and raised by handled mothers were more active than those born and raised by non-handled mothers. Denenberg (1970) has questioned this discrepancy and concluded "I do not believe there is sufficient data available as yet to make any very definitive statements" (p86) [5].

Since this early work, it has become clear that the effects of the interaction between mother and offspring are not unidirectional, that is do not just pass from mother to progeny. It has been demonstrated that each member of this pair influences the other's emotional behaviour (Denenberg 1966). In general, females who raised pups showing low emotionality tend to be less emotional than those which reared high emotional pups. Handling of the female in her infancy tends to influence offsprings' reactivity to further manipulations (Denenberg, Karas, Rosenberg and Schell 1968; Porter and Wehmer 1969) as well as having profound effects on the both the exploratory (Porter and Wehmer 1969; Denenberg 1970) and emotional (Denenberg 1969a) behaviour of her offspring. Offspring of rats that have been handled in infancy show physiological differences too. Levine (1967) has shown that rats reared by handled mothers exhibit a reduced steroid response to novel stimuli, when compared with controls reared by non-handled mothers. However, handling the pups before they themselves were weaned tended to obliterate the effects of the experience of the mothers in infancy. This suggests that the development of behaviours

[5] At the time of submission of this thesis there was still no progress with respect to this issue.

induced by handling are interactional, rather than additive, an hypothesis that has been explored more fully by Denenberg (1982).

More recently, Morse (1979) has investigated the postnatal component of the behavioural transmission of emotional traits to offspring, by rat mothers handled in infancy. In his doctoral dissertation, an original population of emotional and nonemotional female rats was created through infantile handling. These females were then bred and their offspring cross fostered to control for nonbehavioural trait transmission. When mature this second generation was tested and offspring behavioural profiles were compared with those of their mothers. This comparison demonstrated parent and offspring profiles to be virtually the same, a finding Morse attributed to behavioural transmission mediated by "behaviours in the maternal-young lactation period relationship". In particular, differences involving stimulation of the pups were found in the two types of mothers, such that the maternal behaviour of nonemotional mothers produced an environment involving higher stimulation for the pups than that provided the pups of emotional mothers. These findings clearly support Denenberg and Whimbey's (1963) speculations concerning the possible mediating factors involved in the transmission of maternal experiences across to their offspring, namely that "modifications were mediated through... the postnatal mother-young interaction" (p1193).

As with the prenatal stress manipulations (Wehmer, Porter and Scales 1970) handling has also been investigated across two generations, and has been found to affect both activity and weaning weights of grandpups. Specifically, Denenberg and Rosenberg (1967) [6] have shown that descendants of non-handled grandmothers were more active than descendants of handled [7] grandmothers, if their mothers had been reared in maternity cages between birth and weaning. Interestingly, exactly the opposite pattern was obtained if their mothers had been reared in a free environment during infancy. Furthermore, weanlings whose grandmothers were not handled weighed significantly more than those whose grandmothers had been manipulated. In their discussion, Denenberg and Rosenberg point out that this effect only manifests itself when maternal experi-

[6] This experiment is described in more detail in chapter six.
[7] Handling occured between day 1 and day 20 whilst the grandmothers were pups.

ences included a degree of enrichment, pointing out that "the interactive nature of the variables should be emphasized: if we had merely taken the female offspring of handled and non-handled grandmothers and maintained them in standard laboratory caging conditions from birth until adulthood most of the significant findings would have disappeared" (p550). The nature of the mechanisms underlying these effects is not known, although the authors speculate that non-genetic transfer of information is taking place. As they point out "both handling and free environmental experience have behavioural and biological effects. These effects could act through changes in grandmaternal or maternal behaviour or through physiological changes which would affect the developing foetus or modify the milk supply of the grandmother or mother" (p550). What is interesting from the point of view of the present thesis, however, is that minimal environmental manipulations can have such long lasting effects.

Thus, from the studies presented so far, it can be seen that manipulations prior to conception, have clear effects on both offspring and grandoffspring of animals subjected to both punitive and minimal environmental experience. This is particularily relevant to the present thesis, which employs differential maternal environments prior to pregnancy, a procedure which could only indirectly affect future generations. However, manipulation of the mother by exposing her to differential environments has not just been confined to the period prior to conception in the literature, but has also occured during pregnancy and in the perinatal period. Evidence from alternative maternal manipulations relevant to both of these time periods will be outlined in the following sections.

3:3 PRENATAL INFLUENCES

Recently, increased knowledge of normal embryogenesis and of teratogenesis has made it clear that the developing organism is susceptible to changes in its prenatal environment. Indeed, over the past three decades reviews of the literature gathered from diverse sources (Archer and Blackman 1971; Copans 1974; Ferreira 1965; Herrenkohl 1983; Joffe 1965a; 1969a; 1969b; 1978;

1982; Montagu 1962; Smotherman and Robinson 1986; Ward 1984; Ward, Orth and Weisz 1983) have delineated prenatal influences [8] as an area of research in its own right (Joffe 1969b).

As outlined in the introduction to this chapter in general only those studies which have employed a non-toxic or non-pathological manipulation and which fall under the general title of "maternal stress" will be reviewed. Research which fits this criterion and which falls within the framework of "prenatal influences" has come to be called "prenatal stress" (Joffe 1969b) and provides the focus of this particular section [9]. It should be noted, however, that occasionally literature in which the manipulation imposed upon the mother which could be considered "harmful" (such as shock or heat) will also be considered in this review. This is for two reasons. Firstly, seminal papers which have initiated a particular line of investigation but may have themselves employed toxic or pathological manipulations provide an historical framework for the more obviously relevant reasearch, so are included in this review section. Secondly, as the boundaries between that which constitutes "prenatal stress" as opposed to "pathological stress" are sometimes difficult to delineate, the author has occasionally incorporated research from the latter category in order to present as complete a picture as possible.

Historically, investigation of prenatal stress effects in animals stemmed from early reports in the human literature, which suggested that prenatal psychological stress influenced the behaviour of offspring. Within this literature (Reviews: Montagu 1962; Joffe 1969b; Copans 1974) various aspects of behaviour have been measured including neonatal activity and crying (Ottinger and Simmons 1963; 1964), the occurrence of tics (Pasamanick and Kawi 1956), reading ability (Kawi and Pasamanick 1959), temperamental impairment (Stott 1959), mental deficiency (Pasamanick and Lilienfeld 1955) and childhood behavioural disorders (Pasamanick and Lilienfeld 1955; Pasamanick, Rogers and Lilienfeld 1956). The causal agents were generally ill defined, for exam-

[8] A definition of prenatal influence would be the area of research which relates events prior to birth to effects on the postnatal behaviour and anatomy of organisms.

[9] It should also be mentioned, however, that a wealth of literature investigating the effects of manipulating the internal environment of the mother also exists and studies examining the effects of exposing pregnant females to radiation, anoxia, audiogenic seizures, nutrition, disease and drugs on the survival rate, morphology and behaviour of their offspring are constantly appearing in the relevant journals. Comprehensive accounts of these findings can be found in Montagu (1962) and Joffe (1969b; 1982).

ple "maternal complications" or "maternal anxiety" (Archer and Blackman 1971). Because these studies provided limited but inconclusive evidence that prenatal psychological stress [10] influenced offspring behaviour and because of the methodological problems involved in investigating this phenomenon under controlled conditions in human subjects (Joffe 1969b pp232-236), a number of experimenters have studied the behavioural and physiological effects of prenatal stress in *animal* subjects, most typically using rodents. It is these studies which form the basis of the present review, the structure of which owes much to the clear analysis presented by Archer and Blackman (1971). Prior to embarking on this review, however, there are some methodological problems associated with this type of research which require elucidation.

3:3:1 Methodological Problems

There are three types of methodological problems which should be considered. The first concerns the nature of controls used, be they different sorts of nonstressed conditions (Archer and Blackman 1971), or control of prenatal and postnatal variables (Joffe 1969b), the second, the question of the littersize (Chapman and Stern 1979) and the third, whether the results of these studies are in all cases solely attributable to maternal changes induced by the psychological stressors (Archer and Blackman 1971).

A number of different control treatments have been employed in the experiments to be described in this review. For example, taking the first form of psychological stressor to be used in this literature, conditioned avoidance learning, Thompson and his colleagues (Thompson 1957a; Thompson et al 1962; Thompson and Quinby 1964) employed untreated controls, whereas Hockman (1961) used controls which were handled daily. This latter procedure itself has effects on the offspring (Ader and Conklin 1963; Ader and Plaut 1968), thus Hockman's investigation is not stricly comparable with those of Thompson and his co-workers. As will become clear in the following pages

[10] N.B. Archer and Blackman (1971) have defined a psychological stressor as "a situation which although not physically harmful in terms of causing tissue damage, evokes hormonal changes characteristic of the stress response originally described by Selye for physical stressors" (p195). (Selye (1950) defined stress as a nonspecific hormonal change which occured in response to physically harmful stimuli.)

this lack of consistency in the use of control procedures is prevalent in most prenatal paradigms. This led Archer and Blackman (1971) to conclude "that many of the prenatal stress studies are not directly comparable with one another, because different "control" groups have been used by different investigators: in fact, in some cases the comparison is between a treated and an untreated group, whereas in others it is between 2 (sic) differently treated groups" (p197).

Control of both the prenatal and postnatal variables are also important methodological considerations (Joffe 1969b). The first difficulty, relating to prenatal maternal effects, is that in any experiment using different subjects or strains of subjects there may be differences in a number of prenatal variables (such as genetic variables) in addition to environmental ones. For example, different females provide different intra-uterine environments and as Joffe (1969b) points out "mothers of a given strain will conceive foetuses only of that strain. Hence the role of the maternal genotype cannot be assessed separately, since both the maternal and foetal genotypes vary at the same time" (p26). Although a technique exists which avoids this problem, namely reciprocal crossing [11], few studies have employed this procedure. A second and perhaps more confounding problem in prenatal stress research concerns the postnatal environment of the offspring. In its simplest form an experiment on prenatal stress requires the application of some form of treatment to females during pregnancy while an equivalent group is left untreated. If the behaviour of the offspring of the treated mothers differs from that of the controls, this difference can be attributed to the treatment applied. However, it cannot be determined whether the effects were transmitted to the offspring before or after birth, since the effects of the treatment of the mother may well persist after the birth of her offspring, even if the treatment itself terminated at birth. The very possibility of any postnatal effects should be sufficient reason to require control of postnatal variables in prenatal stress experiments, however, as will be seen in the section on perinatal influences later in this chapter, experimental demonstrations of the effects of postnatal

[11] Reciprocal crossing is where males of one strain are mated with females of another strain and males of the second strain with females of the first strain. If inbred strains are used in the reciprocal cross, females of both strains will then carry offspring of like genotype since the crossbred progeny of two inbred strains should be uniformly heterozygous. Any differences between the the two sets of progeny (in the direction resembling the female parent) must then be due to prenatal maternal effects, assuming that is, adequate controls for postnatal effects.

maternal variables on offspring behaviour (such as style of mothering) make it imperative to exclude the role of postnatal factors, before effects of experimental treatments on offspring can be regarded as prenatally mediated. Control of postnatal variables can take several forms and has been discussed in detail by Joffe (1969b pp 21-26). Suffice it to say at this point that in most cases, a form of fostering or cross fostering is probably the most efficacious method of control and for further information on postnatal variables, the reader is directed to the following section on perinatal influences (section 3:4).

The second class of methodological problems, which is related to the issues just discussed, concerns littersize effects, a factor which may influence both the profusion of stress effects and interactions and the lack of consistency across studies. Chapman and Stern (1979) have pointed out that "uncontrolled litter effects may have contributed to the many complex treatment effects obtained in prenatal stress studies" (p258) and further remark "that the unwary investigator who uses several animals from each litter as subjects and then analyses data from prenatally treated offspring without regard to their litter membership risks obtaining significant treatment effects when none actually exist" (p263). The increased likelihood of finding a statistically significant effect when the litter variable is ignored is due a) to a constriction of error variance and b) to a substantial increase in the degrees of freedom of the error term when several subjects per litter are used (Abbey and Howard 1973). According to Chapman and Stern this neglect of the litter variable is common in the prenatal stress literature and that examination of said literature "reveals the possibility that litter effects may have accounted for significant treatment effects" (p264). This implies that reports not employing the correct methodology are not necessarily generalisable and futhermore, that prenatal effects, when present, are likely to be subtle rather than robust.

The third class of methodological problems concerns whether the prenatal stress experiments have been adequately controlled for influences on offspring behaviour which might be transmitted by means other than through maternal changes. These other possible ways in which prenatal stress

might influence offspring behaviour are first by direct effects on the foetus and secondly, as outlined above, through postnatal maternal behaviour induced by the prenatal stress.

With regards to the first possibility, it has been pointed out that certain of the techniques employed in the prenatal stress studies could act directly on the foetus (Archer and Blackman 1971), for example, electric shock used in the conditioned avoidance procedure (Thompson and Quinby 1964) or as a procedure on its own (Sobrian 1977), tilting stress (DeFries 1964; DeFries and Weir 1964) and any procedure involving a sound or heat stimulus (Thompson 1957b; Ward 1984). This has led some researchers to question the efficacy of these techniques as prenatal stressors. However, although these methods do not fully exclude possible direct effects, at present the importance of these effects is unknown. Another possible way in which a prenatal treatment might influence offspring behaviour is through changes in maternal behavour caused by stressor effects on the mother. As mentioned earlier, control for these effects has typically involved fostering. Indeed, Joffe in his doctoral dissertation (1965d) maintained that studies lacking such control were of little value. Although the inclusion of postnatal controls is preferable in prenatal stress experiments, Archer and Blackman (1971) have reviewed this literature and point out that "the studies which used controls for postnatal effects generally revealed no differences between fostered and non fostered offspring in the general direction of their response to prenatal stress" (p201) and conclude that "Joffe's position may be regarded as excessively cautious" (p201).

3:3:2 Prenatal Stress Manipulations

Having considered the principal methodological difficulties involved in the experimental studies of prenatal psychological stress, this section will detail the effects of the main manipulations that have been employed in the literature. A cursory glance at any review of this area suggests that a number of qualitatively different procedures have been used to induce prenatal stress, one of the earliest being that of Thompson and Sontag (1956) who examined the effects of maternal audiogenic seizures from day 5 to 18 of gestation on offspring water maze performance,

activity, weight and littersize. Offspring of prenatally stressed animals were significantly slower in watermaze learning than controls, but did not differ on any of the other dependant variables. However, whether this procedure can be considered as a prenatal stressor as defined by Archer and Blackman (1971) is questionable, as it is not entirely clear if the effects are mediated through changes in maternal hormones. In their discussion Thompson and Sontag include "maternal blood chemical changes due to shock" (p456) as one way in which audiogenic seizures in pregnant animals might affect their offspring, but they also suggest that foetal anoxia resulting from contraction of the uterine arteries might have produced the effects. Consequently, the most commonly cited *original* prenatal stress experiment in the literature is that of Thompson (1957a), who employed a conditioned avoidance manipulation.

Other than avoidance conditioning, a technique that Joffe (1978) has described as a psychological stressor as it does not subject animals to painful or physically stressful events during pregnancy, two other categories of maternal manipulation have been employed that fall within the fairly wide boundaries of maternal stress delineated earlier. These include physical stressors, procedures which appear to be physically stressful or to markedly increase levels of physical stimulation relative to controls, and painful stressors, procedures which include components that are either definitely or probably painful. Table 3:2 details the manipulations which fall within these main categories, findings of which will outlined below.

Psychological Stressor	Avoidance Conditioning	
Physical Stressor	Handling	
	Crowding	With/Without Injection
	Aversive Procedures	Swimming/Tilting/Noise
		Swimming/Noise/Light
		Noise/Light
		Bright Light
		Heat/Light
	Immobilisation	Restraint/Heat/Light
		Restraint/Heat
		Restraint/Light
Pain Stressor	Audiogenic Seizure	
	Conflict	
	Shock	Shock/Light

Table 3:2 Categories of prenatal stressors.

a) PSYCHOLOGICAL STRESSORS

The only psychological stressor in the literature which is relevant to the present work is avoidance conditioning. This procedure typically involves training females prior to pregnancy to avoid shock on presentation of a signal by crossing to the other side of a two compartment shuttle box. Females are then mated and are re-introduced to the shuttle box during gestation, during which time the noxious shock stimulus is withheld. Variations of this general paradigm constitute the most commonly employed technique for imposing prenatal stress and since Thompson's early work (1957a) there have been at least twenty-two studies using this method (Doyle and Yule 1959a; 1959b; 1959c; Hockman 1961; Ader and Belfer 1962b; Thompson and Quinby 1962; 1964; Thompson, Watson and Charlesworth 1962; Joffe 1965a; 1965b; Morra 1965a; 1965b; Bell, Hendry and Miller 1967; Lamp 1967; Porter and Wehmer 1969; Archer and Blackman 1970; Hutchings and Gibbon 1970; Smith, Heseltine and Corson 1971; Smith, Joffe and Heseltine 1975; Masterpasqua, Chapman and Lore 1976; Joffe 1977; Rohner and Werboff 1979).

The most commonly used test within this literature has been the open field with all but one (Bell et al 1967) of the studies employing this apparatus. As with the enrichment literature, interpretation of the open field measures has varied between experimenters, with ambulation

scores being construed as a measure of emotionality (Thompson 1957a; Ader and Belfer 1962b; Thompson and Quinby 1964; Morra 1965b) or exploration (Masterpasqua et al 1976). Overall, however, open field ambulation scores have been remarkably consistent despite a variety of procedural differences between experimenters, most results demonstrating that offspring of prenatally stressed females have decreased ambulation scores when compared with controls. Only three studies have reported the opposite (Thompson, Watson and Charlesworth 1962; Porter and Wehmer 1969; Masterpasqua, Chapman and Lore 1976) namely that offspring of stressed animals are more active, with five studies (Joffe 1965a; 1965b; Lamp 1967; Archer and Blackman 1970; Joffe 1979) reporting no significant differences between the groups. This has lead Ader and Belfer (1962b) to conclude that "these results provide some indication that prenatal maternal anxiety can influence emotional behaviour of the offspring"(p715) further corroborated by Thompson and Quinby (1964) who report that "changes are in the direction of increased emotionality as indicated by depression of open field activity" (p370).

Latency of ambulation has also been measured in a few studies. Ader and Belfer (1962b), Hockman (1961) and Thompson et al (1962) reporting no significant differences as a function of prenatal treatment, but Thompson (1957a) and Morra (1965a) reporting increased latencies in prenatally stressed rats. In contrast Porter and Wehmer (1969) found significantly shorter latencies in their prenatally stressed animals.

Generally no significant differences have been found between the groups with respect to defecation (Hockman 1961; Porter and Wehmer 1969; Joffe 1965a; 1965b; 1977; Smith, Joffe and Heseltine 1975; Masterpasqua, Chapman and Lore 1976). However, Thompson (1957a) and his co-workers (Thompson et al 1962) have reported increased defaecation in the open field as a function of prenatal stress. Some authors have measured other aspects of behaviour in the open field situation, for example Archer and Blackman (1970) observed the number of entries into the inner circle of their open field and found that after both one and ten minutes' exposure to the apparatus, number of entries were significantly less in their prenatally stressed offspring than in

untreated controls. In addition, Doyle and Yule (1959a) found a significant increase in freezing behaviour in the open field in prenatally stressed animals. In contrast, Hutchings and Gibbon (1970) reported that their stressed animals spent less time crouching than controls.

Summarising the results of the open field tests, the direction of the behaviour change produced by prenatal stress is in most cases that of decreased ambulation and a tendency towards emotionality in a novel situation. Additionally, as Archer and Blackman (1971) point out "most of the other behavioural tests used in the prenatal stress studies have produced similar results to the open field data" (p231).

Moving on to other test procedures employed in this literature, Thompson (1957a) reported that prenatally stressed animals demonstrated longer latencies than controls in an emergence from home cage test at 30-40 days of age, but by 135 days this significance seemed to have disappeared (Ader and Belfer 1962b). Interestingly Masterpasqua et al (1976) found that their prenatally stressed animals spent a greater amount of time outside their home cages (when tested at 45 days of age) and interpreted this as an increase in exploratory behaviour in these animals. Latency to reach a food reward along a runway has also been measured. In his early work Thompson (1957a) found increased latencies in a runway test as a result of prenatal stress at both 30-40 days and 130-140 days, interpreting this as evidence of increased emotionality in these animals. However, in a later experiment (Thompson et al 1962) he found that the direction of change was a function of the activity of the parental strain, rats from a high-active strain showing increased latencies, whereas those from a low-active strain showed decreased latencies, when compared with controls. Thompson and Quinby (1964) extended this work, when they reported that direction of change also depended on the sex of the offspring, males showing increased latencies as a function of prenatal stress, females showing the opposite, namely decreased latencies.

Not all the tests used in this literature have been concerned solely with the general reactivity of the organism. Several experiments have employed learning paradigms, in particular avoidance conditioning and watermazes, as well as investigating social behaviour and physiological changes.

With respect to performance in a learning task, Thompson et al (1962), Thompson and Quinby (1964) and Morra (1965b) have investigated the effects of prenatal stress on the performance of the offspring in a watermaze. None of these studies revealed any effects of the prenatal treatment with respect to the number of trials required to reach criterion. However, if the animals were retested after two weeks, stressed offspring required significantly more trials to reach criterion than their untreated counterparts (Thompson et al 1962). Time taken for each trial varied according to experimental background, with longer swim times for prenatally stressed animals than controls (Thompson et al 1962; Thompson and Quinby 1964). In contrast, Morra (1965b) found that offspring from more severely stressed mothers learned a watermaze faster than those from less severely stressed mothers. It should be pointed out, however, that Morra's learning situation was procedurally different from that employed by Thompson and his co-workers and the animals differed in age and previous test experience, which may account for the discrepancy in the results. Performance in a complex maze has also been investigated (Lamp 1967), with errors and time per trial being measured. No significant differences were found between the offspring of prenatally stressed and control mothers. However, Lamp (1967) points out that his work employed differences in experimental variables, but does not specify them.

The other sort of learning situation which has been used in this literature is conditioned avoidance itself. Joffe (1965b) reported that prenatally stressed offspring scored significantly more avoidance responses with significantly shorter latencies than control offspring. However, in a later experiment, avoidance conditioning procedures only manifested effects in female offspring (Smith, Joffe and Heseltine 1975) and in this instance, stress significantly decreased avoidances made by the offspring. In a further experiment (Joffe 1977), effects were not found. In this latter paper, however, animals had received saline injections as part of a drug study, a procedure which in itself is stressful and may have caused changes in the "control" animals' behavioural repertoire.

More recently, a variety of social behaviours have also been examined in the prenatal stress

literature, where stress has been induced by prenatal avoidance conditioning. In particular Smith, Joffe and Heseltine (1975) included a social dominance task in their range of test procedures, in which pairs of offspring of control and stressed females, matched for body weight, were housed in hanging cages and fed one 10g pellet of rat food twice a day. Animals were weighed at the end of 7 and 14 days and the percent of body weight loss used as a measure of dominance. Males lost more weight than females, but no significant weight differences were found between the stressed and nonstressed offspring. This finding has been interpreted by the authors in the light of weight losses sustained by stressed and nonstressed animals in a food deprivation task. Specifically they suggest that "the finding that stressed animals lost less weight in a food deprivation task than controls, but not in the social dominance test implies that there must have been differences in the competitive abilities of stressed and unstressed animals. If each each member of a pair (one stressed and one unstressed rat) were obtaining an equal quantity of food in the dominance situation, stressed animals should have lost less weight as they did when caged individually and fed an equal amount of food to that received by nonstressed animals" (p467). Thus it appears that nonstressed animals were obtaining a larger share of the food and were more socially dominant.

The relationship between prenatal stress and the sexual and emotional behaviour of adult male rats has also been examined in the literature (Masterpasqua et al 1976). Beginning at 90 days of age, all males were given three weekly 30 minute sexual tests conducted with ovariectomised estrous females. Number of genital sniffs, mount latencies, number of mounts, intromission latencies, number of intromissions, ejaculation latencies and number of ejaculations were recorded. Additionally, a long term sexual test was also included in the study. At 120 days of age, each male was housed with an adult female rat for 18 days and breeding effectiveness was measured. Results indicated that male offspring of prenatally stressed rats showed low levels of copulatory behaviour, but successfully impregnated their female cagemates.

Physiological changes have also been found in offspring of prenatally stressed animals. Bell et al (1967), for example, found that prenatal avoidance conditioning (CS presentation) adminis-

tered at the time the foetal gut was developing produced increased gastric ulcer susceptibility to immobilisation, when compared with controls which were either offspring of untreated females, or females stressed later in their gestation. Biochemical assays of prenatally stressed offspring have also been undertaken (Rohner and Werboff 1979), with catecholamine concentrations being measured at three different points in the offspring lifespan (birth, weaning and adolescence). Although no significant differences in dopamine concentrations were found in experimental and control pups at birth, adolescent offspring from avoidance conditioned females had significantly reduced dopamine levels in tissue from the corpus striatum when compared to offspring of control mothers. Rohner and Werboff in their discussion suggest that "the dopaminergic system was affected by the prenatal environment and that this in turn affected the activity level of the offspring" (p47).

Finally, weight changes associated with prenatal stress have also been reported in the literature. In particular, Smith, Joffe and Heseltine (1975) have found that stressed offspring were on average 5.1 percent lighter than unstressed pups at birth and that this effect on weight was still present after 21 days of rearing by unstressed foster mothers. These weight differences had disappeared by 42 days, however, there have been other reports in the literature of adult weight differences. For example, Porter and Wehmer (1969) found that adult weight was lower for subjects whose mothers had undergone stress during pregnancy, when compared with control animals. Interestingly, littersizes of experimental animals have also been found to be smaller than those of control animals (Lamp 1967) and in this study maternal behviour was also observed, several of the stressed mothers being found eating their young.

Thus it can be seen that avoidance conditioning during gestation has profound effects on offspring behaviour and physiology. There are however several other findings which are of interest and require elucidation, before moving on to consider the effects of *physical stressors* such as handling and immobilisation on offspring development.

Firstly, several researchers have investigated the effects of differences in the *intensity* of prenatal

stress on the offspring response. Morra (1965b) for example, induced different levels of prenatal stress by exposing his female rats to different numbers of premating avoidance training trials (0, 50, 100 and 200) whilst using a constant number of exposures during pregnancy and found that "greater levels of conditioned stress during pregnancy seemed to result in greater emotionality in the offspring " (p8). Similarly, Thompson and Quinby (1964) induced high and low levels of stress in pregnant rats, by employing different numbers of avoidance training and test trials and different numbers and strengths of shock during training. When the offspring were tested in an open field, it was found that their activity was inversely related to the amount of maternal stress. Conversely, however, offspring latency scores in a watermaze, which were greatly increased by prenatal stress, were more affected by a low level of stress than by a higher level. In their conclusion, these authors state "the extent and direction of effects are dependant in complex ways on the sex of the animal, parental activity levels and intensity of maternal stress" (p371).

As well as intensity of stress, a second variable, the *timing* of the stress has been of interest to some researchers. Morra (1965b) studied the effects of prenatal stress administered in either the first or second half of pregnancy and found that the latter half of pregnancy seemed to be more sensitive to the treatment. In a subsequent study, Bell et al (1967) manipulated the prenatal stress such that it occured either when the foetal gut was developing (Days 6-10 of pregnancy) or at a later stage (Days 11-14). Controls received no treatment during pregnancy. Offspring were tested at 46 days for gastric ulcer susceptibility after 48 hours of immobilisation and those stressed in the earlier period were found to demonstrate significantly more ulcers than offspring from the other two conditions.

As well as timing and intensity of stress, a third variable that has been investigated is the postnatal environment. In particular, Hockman (1961) noted that the stress applied during gestation in his experiments was not by itself sufficient to noticeably affect the offspring but that it "must be supplemented by the cross fostering experience" (p682). However, this finding may well have resulted from a procedural variable particular to Hockman's work, as there are numerous

studies which have reported significant findings without employing any fostering techniques at all (Doyle and Yule 1959a; 1959b; 1959c; Thompson et al 1962; Morra 1965a; 1965b; Bell et al 1967; Hutchings and Gibbon 1970).

Finally, the nature of prenatal stress induced by avoidance conditioning has been compared with other manipulations. Porter and Wehmer (1969) have reported that the "main effects of maternal pregnancy stress upon subsequent offspring open field performance appear much more pronounced than the effects of infantile handling in the offspring" (p24).

To summarise, therefore, psychological stressors have pronounced effects on offspring emotionality, exploration and learning, as well as their physiological development. These effects are complex and altered by the timing and intensity of the maternal manipulation. Psychological stressors are not the only procedures to be employed in this literature, however and in the following pages, the effects of *physical stressors* applied to the mother during her pregnancy on her offspring will be reviewed.

b) PHYSICAL STRESSORS

As can be seen from Table 3:2 (described earlier), physical stressors include handling, crowding, immobilisation and other aversive procedures; there have been numerous studies which have used these manipulations. These are listed in Table 3:3 [12]. Early work in this field concentrated on the effects of prenatal experience on reactivity (Keeley 1962; Lieberman 1963; Ader and Conklin 1963; De Fries 1964; Weir and De Fries 1964; De Fries and Weir 1964; De Fries, Weir and Hegmann 1967), but following Ward's (1972) assertion that prenatal stress both demasculinised and feminised male rats' sexual behaviour, interest shifted to examining this in more detail. Both the behaviour and underlying physiology of sexual behaviour have since been explored, as have maternal behaviours. More recently, some work has also investigated the effects of various

[12] Two additional studies should also be included in this table; the first research by Rojo, Marin and Menendez-Patterson (1985) who used what they term a "low stress", the second the work of Grimm and Frieder (1987) who employed a "mild stress". As no further details were available to the author, these studies cannot be categorised and consequently are included only as a footnote.

stressors on learning ability (Smith, Wills and Naylor 1981; Ryakaszewski 1985; Fride, Dan, Feldon, Halevy and Weinstock 1986;), maturation (Sobrian 1976; Fride and Weinstock 1984; Fride et al 1986) and play (Ohkawa 1987). The most recent publications in this field, however, have concentrated on the interaction of prenatal stress and various drugs such as ethanol (DeTurck and Vogel 1982; DeTurck and Pohorecky 1985; Weinberg 1987; DeTurck and Pohorecky 1987) and caffeine (Pohorecky et al 1989). Furthermore, increasingly sophisticated investigations of the impact of prenatal stress on brain biogenic amines (Peters 1982; 1984; 1988a; 1988b; 1990; Fride and Weinstock 1988; 1989), hormonal sytems (Kinsley and Bridges 1987; Weinberg 1987; Kinsley, Mann and Bridges 1989; Pohorecky et al 1989) and opioid systems (Kinsley, Mann and Bridges 1988a; Insel et al 1990) have also appeared in the literature. The findings from these areas will be described in more detail in the following pages.

Handling	Ader and Conklin 1963 Werboff, Anderson and Haggett 1968 Ader and Plaut 1968 Plaut, Grota, Ader and Graham 1970 Ader and Deitchman 1970 Plaut, Graham and Leiner 1972 Sobrian 1976 Miley, Frank and Hoxter 1981 Smith, Wills and Naylor 1981 Miley, Blustein and Kennedy 1982 Peters 1982 DeTurck and Pohorecky 1985 DeTurck and Pohorecky 1987 Pohorecky, Roberts, Cotler and Carbone 1989
Crowding	Keeley 1962 Lieberman 1963 Chapman, Masterpasqua and Lore 1976 Allen and Haggett 1977 Dahlof, Hard and Larsson 1977 Dahlof, Hard and Larsson 1978 Peters 1982 Peters 1984 Harvey and Chevins 1984 Harvey and Chevins 1985 Moore and Power 1985 Peters 1986a Peters 1986b Moore and Power 1986 Power and Moore 1986 Peters 1988a Peters 1988b Peters 1990
Aversive Procedures	DeFries 1964 Weir and DeFries 1964 DeFries and Weir 1964 DeFries, Weir and Hegmann 1967 Beckhardt and Ward 1983 Fride and Weinstock 1984 Fride, Dan, Gavish and Weinstock 1985 Fride, Dan, Feldon, Halevy and Weinstock 1986 Fride, Soreq and Weinstock 1986 Fride and Weinstock 1987 Fride and Weinstock 1988 Fride and Weinstock 1989

Table 3:3 Summary of those studies which have used experimental manipulations which fall within the category of "physical" stressor.

Immobilisation	Ward 1972 Dahlof, Hard and Larsson 1972 Lane and Hyde 1973 Ward 1974 Herrenkohl and Whitney 1976 Ward 1976 Ward 1977 Moyer, Herrenkohl and Jacobowitz 1977 Whitney and Herrenkohl 1977 Dahlof, Hard and Larsson 1977 Herrenkohl and Politch 1978 Barlow, Knight and Sullivan 1978 Dunlap, Zadina and Gougis 1978 Dahlof, Hard and Larsson 1978 Moyer, Herrenkohl and Jacobowitz 1978 Politch, Herrenkohl and Gala 1978 Chapman and Stern 1978 Herrenkohl 1979a Herrenkohl 1979b Herrenkohl and Gala 1979 Meisel, Dohanich and Ward 1979 Politch and Herrenkohl 1979 Chapman and Stern 1979 Meisel 1980 Gotz and Dorner 1980 Ward and Weisz 1980 Rhees and Fleming 1981 Miley, Frank and Hoxter 1981 DeTurck and Vogel 1982 Burack 1982 Weisz, Brown and Ward 1982 Miley, Blustein and Kennedy 1982 Wilke, Tseu, Rhees and Fleming 1982 Ward, Orth and Weisz 1983 Vom Saal 1983 Orth, Weisz, Ward and Ward 1983 Dorner, Gotz and Docke 1983 Rhees, Badger and Fleming 1983 Miley 1983 Herrenkohl 1983 (Review) Herrenkohl and Scott 1984 Ward 1984 Ward and Weisz 1984 Politch and Herrenkohl 1984 Pollard 1984 Pollard and Dyer 1985 Ward and Ward 1985

Table 3:3 continued

Immobilisation (continued)	Anderson, Rhees and Fleming 1985 Ward and Reed 1985 Rykaszewski 1985 Anderson, Fleming, Rhees and Kinghorn 1986 Kinsley and Svare 1986a Kinsley and Svare 1986b Fleming, Anderson, Rhees, Kinghorn and Bakaitis 1986 Ward, Monaghan and Ward 1986 Kinsley and Bridges 1986 Herrenkohl 1986 Kinsley and Svare 1987 DeTurck and Pohorecky 1987 Weinberg 1987 Kinsley and Bridges 1987 Ohkawa 1987 Kinsley and Svare 1988 McLeod and Brown 1988 Kinsley and Bridges 1988 Kinsley, Mann and Bridges 1988a Kinsley, Mann and Bridges 1988b Lephart, Fleming and Rhees 1989 Kinsley, Mann and Bridges 1989 Insel, Kinsley, Mann and Bridges 1990

Table 3:3 (continued)

Considering first *reactivity*, as with the psychological stressor avoidance conditioning detailed in the previous section, one of the most commonly employed test procedures has been the open field, with a variety of prenatal stressors being employed, including crowding (Lieberman 1963; Chapman, Masterpasqua and Lore 1976; Moore and Power 1986; Peters 1988a; Peters 1988b), handling (Ader and Conklin 1963; Plaut, Graham and Leiner 1972; Sobrian 1976; Pohorecky et al 1989), swimming, tilting and noise (DeFries 1964; DeFries, Weir and Hegmann 1967), swimming, tilting and exposure to an open field (Weir and DeFries 1964; DeFries and Weir 1964), noise and light (Fride, Dan et al 1986) and restraint (Meisel, Dohanich and Ward 1979; Chapman and Stern 1979; Rykaszewski 1985). Results reflect the procedural diversities, with no significant differences in activity emerging between offspring groups when the prenatal stress was handling or noise and light, but stressed offspring being more active than controls when their mothers had been crowded during their pregnancies. Sex effects also manifest themselves, with significant differences emerging between female offspring of prenatally restrained and control

females. DeFries and his colleagues, who employed swimming, tilting, noise or open field exposure did find differences in activity in their groups, however, these interacted with strain of animal. In particular, offspring of stressed animals from a highly active strain of mice (C57BL/6J) were less active in the open field test (DeFries and Weir 1964), whereas the converse was found in a low activity strain (BALB/CJ Weir and DeFries 1964). Finally, in one experiment (Sobrian 1976) both handling and footshock were employed as prenatal stressors and were found to influence open field ambulation. However, the author does not specify the direction of influence, only that "the direction of the change was dependant on the level of stress to which the pregnant female had been exposed" (p6423-B).

Measures of defecation in the open field have typically revealed no significant differences between stressed and control groups, the one exception being Ader and Conklin (1963), who reported that offspring of prenatally handled animals defecated significantly less than control offspring. This finding is particularily interesting, as it contrasts with the greater emotionality effected by prenatal maternal anxiety induced by avoidance conditioning. Indeed, as Ader and Conklin point out "to the extent that high emotionality may be considered maladaptive, such results serve to contradict any orientation or expectation that only deleterious effects can result from prenatal manipulation" (p412).

Lower emotionality as defined by defecation levels, was supported in Ader and Conklin's research by the finding that prenatally stressed animals were more likely to approach the centre of the open field [13]. This lack of fear has also been reported by Moore and Power (1986) who employed a crowding procedure as their prenatal manipulation [14].

Other than the open field test, reactivity has also been measured in tests of emergence (Keeley 1962; Ader and Conklin 1963; Chapman and Stern 1979; Chapman, Masterpasqua and Lore 1976) and in the animals' reactions to handling (Ader and Plaut 1968; Plaut, Graham and

[13] The amount of time spent in the centre of the open field is often considered to reflect levels of emotionality, less time indicating higher emotionality Walsh and Cummins (1976).

[14] Although more recently, Fride and Weinstock (1989) have reported that when noise and light are employed as the prenatal stress procedure, offspring of stressed dams demonstrate increased anxiety related behaviour.

Leiner 1972). As with the open field, the nature of the prenatal stressor has an effect on the offspring response. For example, Chapman and Stern (1969) who employed restraint, heat and lights as stressors found no significant differences between their groups, although, as they point out, very few rats emerged from their home cages during their ten minute test trials. Moreover, other measures taken in this study, including freezing behaviour, defecation for non-emergers and rearing frequencies, all failed to reveal any differences between the groups. Crowding, on the other hand, has an effect as a prenatal stressor. Both Keeley (1962) and Chapman et al (1976) reported differences between their experimental and control groups, with the offspring of crowded animals being the least likely to emerge. The latter experimenters explored this further by manipulating the degree of crowding and found that significantly fewer offspring of mothers exposed to high density crowding emerged above the top of their cage when compared with low and medium density offspring. Handling has also been employed as a prenatal stressor in this literature. Ader and Conklin (1963) found a significant group effect, mediated by an interaction with the type of postnatal experience and sex of the animal. Among males, no differences were found between non-crossfostered handled and control groups, whereas crossfostered groups of prenatally handled animals emerged significantly sooner than controls. Amongst females it was the crossfostered groups that did not differ significantly, but the non-crossfostered offspring of handled mothers emerged significantly sooner than controls.

In the two studies exploring the reaction of prenatally stressed and non-stressed offspring to being handled, interactions between the postnatal experiences of the offspring have also been found. In particular, Ader and Plaut (1968) reported that in group housed animals there was a lower incidence of startle responses and significantly less resistance to being picked up in the offspring of handled as compared to control females, but that these differences only occurred in those animals that had previously been subjected to some kind of stimulation. Plaut, Graham and Leiner (1972) included perinatal housing of some litters with a virgin female "aunt" and found that both prenatal handling and rearing with aunts affected scores of female offspring in a reaction to handling test. More specifically, handling increased the number of startle responses

among females reared with aunts and among the female offspring of nonhandled mothers, a greater proportion of animals reared with aunts exhibited resistance to being picked up when compared to rats reared without aunts, however, this resistance was prevented in animals whose mothers has been prenatally stressed.

As mentioned earlier in this section, investigations of the effects of prenatal experience have extended to a variety of behaviours other than reactivity. In particular, researchers have considered offspring performance in learning tasks, as well as examining sexual, maternal and aggressive behaviours. Furthermore, maternal stressors have been shown to influence offspring anatomy, neuroanatomy and neurochemistry as well as circulating hormones. These findings will be discussed in turn in the remainder of this section. For the sake of simplicity, as there are quite a few studies to be detailed in this section, information will be further categorised into two subsections according to whether the prenatal stress affects offspring behaviour or physiology.

Offspring Behaviour

With respect to learning, both a variety of stressors and test apparatus have been employed, with few significant main effects. Werboff, Anderson and Haggett (1968), for example, handled pregnant C57BL/6J mice twice daily for five minutes throughout gestation and tested offspring in a water runway at 45 and 100 days of age. No significant treatment effects were observed, but it was evident that prenatal handling consistently resulted in differential effects on male and female offspring. Male offspring of handled mothers took longer to escape from the runway than controls, the converse being true of the female offspring. Sex differences were also noted by Meisel, Dohanich and Ward (1979), in offspring of Sprague-Dawley mothers restrained and exposed to bright lights three times a day from Day 14-21 of gestation. At approximately 80 days, offspring received six daily sessions of avoidance training, with females demonstrating significantly more avoidance responses than males. In this instance, however, no significant treatment by sex interactions emerged.

More recently, however, Fride et al (1986) have found significant prenatal stress effects in an avoidance conditioning task, qualified by a significant sex by treatment interaction. Acquisition of active avoidance was facilitated in female but reduced in male prenatally stressed offspring. These authors suggest "that random prenatal noise and light stress may cause impairment of development of hippocampal function which lasts into adulthood. This impairment is manifested as an increase in vulnerability and a decrease in habituation to stressful stimuli" (p681). Whether this prenatal stress effect on offspring learning depends on the nature of the stressor, however, is one question which warrants further consideration. For example, Rykaszewski (1985) in her doctoral dissertation, investigated the effects of heat, light and restraint from Day 1-21 of gestation on offspring avoidance conditioning and found no differences between her groups. In a second experiment, she compared prenatally stressed and control males on the acquisition of a conditioned emotional response (CER) and found again, no significant effects of the prenatal stressor. This work is in contrast with that of Fride et al (1986).

One study which has considered the nature of the stressor in some detail, and over six learning tasks, is that of Smith, Wills and Naylor (1981). These authors employed two prenatal stressors, handling and avoidance escape training and found that the nature of the stressor did influence offspring performance. At 60 days, offspring began training. On the first day the operant level of bar pressing was measured by placing each rat in a Skinner box for 30 minutes. Offspring were then placed on a 24-hour food deprivation schedule and on the second day magazine training began. After the rats were magazine trained, bar press training began on a continuous reinforcement (CRF) schedule, training criterion being 50 bar presses in a 30 minute trial. Following this, discrimnation training was undertaken. Number of sessions required to magazine train the animals revealed significant differences between the groups, handled animals taking longer than a control group. There was also a significant difference between the groups on the number of sessions required to acquire the bar press response, avoidance conditioned (AC) animals taking considerably longer to train than either the handled or control groups. Significant differences also emerged with respect to the mean responses per minute during acquisition training, control

and AC animals performing at a significantly faster rate than handled offspring. No differences emerged between the groups with respect to number of sessions required to achieve criterion on the discrimination task. Following Skinner box training, animals were then trained on a simple maze and in a runway. There was a significant difference found between the groups on the number of errors made in running the maze, the control group performing significantly better than the stressed groups. No differences in running time in the runway were found. These results indicate that stresses experienced during pregnancy have an effect on offspring in complex learning situations and that the nature of the stressor is an important variable in the way effects are mediated.

As well as affecting offspring reactivity and learning performance, prenatal (physical) stress has also been found to alter offspring sexual behaviours (Ward 1974; 1984; Herrenkohl 1983; Ward and Ward 1985). The first study to detail this phenomenon was published in Science in 1972 by Ingeborg Ward, who reported that prenatal stress induced by physical restraint of the pregnant mother under bright lights both feminised and demasculinised sexual behaviour in male offspring. In particular, Ward found that for the most part, prenatally stressed Sprague Dawley male rats did not attempt to copulate and following castration, averaged three times as many lordotic responses as the control group. Interestingly, however, this feminised behaviour was qualitatively different from that exhibited by female rats. For example, the prenatally stressed and castrated males did not solicit, nor did they display darting or ear wiggling, behaviours typical of estrous females. On the other hand, they did not resist being mounted and displayed high quality lordotic responses. These data Ward suggested, supported "the hypothesis that exposure of pregnant rats to environmental stressors modifies the normal process of sexual behaviour differentiation in male fetuses by decreasing functional testosterone and elevating androstenedione [15] levels during perinatal development" (p83). In Ward's experiment, offspring were stressed pre and postnatally. The postnatal stress consisted of placing each pup into a plastic ice cube tray mounted on a vibrating rack and stress was effective only during prenatal sexual differentiation.

[15] A less potent androgen than testosterone.

Since this early work, handling has also been employed as a postnatal stressor (Ward 1974; 1976) with no effects. More recently, however, the nature of the postnatal *environment* per se has been investigated (Dunlap, Zadina and Gougis 1978; Ward and Reed 1985) and has been found to interact with the prenatal stress effects. Results demonstrate that rearing the animals in isolation, as compared to a social condition, more severely disturbs stressed males' copulatory behaviour.

Since Ward's seminal work (1972), numerous studies have reported that prenatal stress both demasculinises (Ward 1974; 1976; Allen and Haggert 1977; Dahlof, Hard and Larsson 1977; Dunlap, Zadina and Gougis 1978; Gotz and Dorner 1980; Rhees and Fleming 1981; Burack 1982; Dorner, Gotz and Docke 1983; Rhees, Badger and Fleming 1983; Harvey and Chevins 1984; Ward and Reed 1985) and feminises (Dahlof, Hard and Larsson 1972; Ward 1974; Herrenkohl and Whitney 1976; Ward 1976; Ward 1977; Whitney and Herrenkohl 1977; Dahlof, Hard and Larsson 1977; Rhees and Fleming 1981; Dorner, Gotz and Docke 1983; Politch and Herrenkohl 1984; Ward and Reed 1985) male sexual behaviour. Furthermore, although most of the work has used Sprague Dawley rats, effects have also been found in Wistar rats (Dahlof, Hard and Larsson 1977; Dorner, Gotz and Docke 1983), C56BL/6J mice (Allen and Haggett 1977), TO mice (Harvey and Chevins 1984) and CD1 albino mice (Politch and Herrenkohl 1984). Only one study (Chapman, Masterpasqua and Lore 1976) has reported no significant differences between stressed and control offspring in either a test for male sexual behaviour, or in a fertility test. In this experiment the prenatal stressor employed was crowding and the authors speculated in their discussion that crowding might not be as severe a stressor as physical restraint (Ward 1972) or the blocking of a previously learned avoidance response (Masterpasqua, Chapman and Lore 1976). However, more recently, crowding has been found to demaculinise prenatally stressed animals' sexual behaviour (Allen and Haggett 1977; Dahlof, Hard and Larsson 1977; Harvey and Chevins 1984) thus refuting Chapman et al's explanation, whilst to further confuse the issue, Rojo et al (1985) have reported that low levels of prenatal stress may facilitate rather than inhibit male sexual behaviour. What does appear to be consistent in this literature, is that the nature of

the stressor is an important factor in determining the behavioural response. However, quality of stressor (whether it be mild or severe) does not appear to be correlated with type of behavioural response.

Other than crowding, physical restraint coupled with light or heat and avoidance conditioning, malnutrition and adrenocorticotrophic hormone (ACTH) injections have also been employed as stressors (Rhees and Fleming 1981; Harvey and Chevins 1984; McLeod and Brown 1988) and have been found to affect masculine behaviour. In particular, Rhees and Fleming (1981) compared the effects of nutritional stress, restraint illumination and heat and ACTH administered during the third trimester of gestation and found that compared to control males, male copulatory behaviour was severely impaired in all three experimental groups. The prenatally stressed animals showed a significant reduction in the cumulative percent ejaculating and an increase in the number of intromissions prior to the first ejaculation. When tested for female behaviour, all three treatment groups displayed a significantly greater lordosis quotient than the control males. Harvey and Chevins (1984) also treated dams with ACTH during pregnancy and found that stressed offspring demonstrated fewer mounts and intromissions than controls and had longer intromission latencies, whilst heat and restraint alone (McLeod and Brown 1988) although not influencing number or frequency of ejaculations, did produce offspring with longer intromission latencies as well. So it appears that the demasculinisation and feminisation of males, first reported by Ward (1972) can be induced by a variety of prenatal stressors.

One obvious question to arise from this work, is how are these effects mediated? One hypothesis forwarded by Ward et al (Ward and Weisz 1984; Ward 1984) is that the etiology of this syndrome stems from the same hormonal mechanism(s) that underlies sexual differentiation in both *normal* males and females. Normal masculinisation and defeminisation of sexually dimorphic behaviours requires exposure to adequate amounts of androgenic steroids during specific stages of perinatal development. Ward and Weisz (1984) have suggested that the sexually aberrant behaviours in prenatally stressed animals may result from a stress-induced alteration of the testosterone surge

during days 18 and 19 of gestation. Furthermore, it is becoming increasingly more likely that the aromatization of androgen to estrogen may be required for proper masculinisation of the nervous system, at least in the rat. Prenatally stressed rats have significantly lower levels of brain aromatase activity on days 18, 19 and 20 of gestation than do control animals (Weisz, Brown and Ward 1982). The possible contribution this abnormality makes to the behavioural syndrome shown by the males remains to be elucidated (Ward 1984) although as will become apparent in the following pages, increasingly the interaction of endocrines and neurotransmitters are being implicated as the biological correlates of the functional differences in prenatally stressed and control offspring.

Other than copulatory behaviour, Simon and Gandelman (1977) using a regime of ACTH treatment in pregnant mice have reported the depression of another androgen dependent and sexually dimorphic pattern of behaviour, that of intermale aggression. Of more relevance to the present thesis, however, is the work of Harvey and Chevins (1985) and Kinsley and Svare (1986a; 1987) who employed environmental stressors. Harvey and Chevins found that chronic crowding during pregnancy significantly impaired the expression of both attack and threat responses of adult male offspring in mice. This lack of masculinisation, these authors suggested "could reflect a failure of development in the brain region concerned with aggressive behaviour, or a failure in development of the pituitary-gonadal system" (p91). As the latter is ultimately under hypothalamic control, a developmental defect in the central nervous system is implicated in any case and according to the authors "the question resolves itself to whether the defect is neurobehavoiural, neuroendocrine or both" (p91).

Kinsley and Svare employed the more traditional stressor, restraint and heat, and found that prenatal stress significantly reduced intermale aggression as measured by percentage of animals fighting and number of attacks in Rockland-Swiss albino mice (Kinsley and Svare 1986a). These behavioural changes are thought to be related to alterations in fetal testosterone exposure (Ward and Weisz 1980; 1984), disruptions in aromatizing enzyme activity (Weisz, Brown and Ward

1982) and/or changes in the morphology of the sexually dimorphic nucleus in the pre-optic area (Anderson, Rhees and Fleming 1985). In a second experiment Kinsley and Svare (1987) investigated the degree to which this aggressive behaviour was mediated by genotype and found that prenatal stress increased aggression in C57BL/6J mice, but not in DBA/2J animals. The mechanism(s) responsible for this strain dependent variation is unknown at the present time.

Finally, pup killing and maternal behaviours in prenatally stressed males have also been investigated (Miley, Frank and Hoxter 1981; Vom Saal 1983; Kinsley and Bridges 1986; McLeod and Brown 1988). Considering Miley et al's (1981) paper first, restraint and intense illumination applied prenatally completely suppressed rat-pup killing when compared to levels of pup-killing typically reported in normally reared males (Rosenberg et al 1971). However, the nature of the stressor has profound effects on this behaviour, as offspring of animals handled during gestation, for as little as three minutes a day, killed pups rapidly. Moreover, the impact of prenatal stress on male infanticide behaviour is not species specific, Vom Saal (1983) reporting that offspring of stressed mice were less likely to kill pups and more likely to show parental behaviour than controls. The only other investigation of infanticide (McLeod and Brown 1988) in which rat dams were prenatally stressed by light, heat and restraint did not find any differences between offspring of stressed and control mothers, but did note that if reared from weaning with a non-stressed female no evidence of infanticide emerged at all. These authors concluded that postnatal rearing condition was more important than prenatal stress in suppressing infanticide. It may well be that prenatally stressed males reared together, with their more "feminised" behaviour inhibit each others' infanticidal tendencies, given that inhibition of infanticide in normal adult male rats also occurs after cohabitation with a normal pregnant female (Brown 1986).

With respect to maternal behaviour, a variety of indices have been measured. For example, Miley et al (1981) included (rat) pup retrieval, grooming and licking, crouching over the pups and nest building in their study, whilst McLeod and Brown (1988) measured latency to show parental behaviour, frequency of parental behaviour, frequency to touch and sniff pups and nest building

behaviours. In their study, Kinsley and Bridges (1986) measured what they term full maternal behaviour (retrieval and grouping of and crouching over pups). Generally prenatal stress has been found to increase male maternal behaviour (McLeod and Brown 1988; Kinsley and Bridges 1986) although it should be noted that in Miley et al's (1981) study only pup retrieval revealed significant differences between the groups, offspring of control mothers retrieving significantly faster than the experimental groups. As with infanticide, the effect is not just confined to rats, prenatally stressed male mice also displaying more parental behaviours than control animals (Vom Saal 1983).

To summarise, sexually dimorphic aspects of behaviour are particularily vulnerable to environmental insult during pregnancy, at least in male offspring of altricial rodent species. Whether female offspring are similarily at risk is less clear, as there are conflicting results in the literature, as will become apparent in the following pages.

The first investigation of the effects of prenatal stress on female offsprings' sexual behaviours was that of Ingeborg Ward in 1974. In her study the prenatal stressor consisted of restraint in a Plexiglas tube and exposure to 200 foot-candles of light from day 14-21 of gestation. Female offspring were primed with testosterone propionate (TP) and after five weeks of daily TP injections and biweekly testing with an estrous female, no differences could be detected in the number of incomplete and complete copulations emitted by responding animals in the stressed and nonstressed groups. However, prenatal stress did reduce the percentage of females capable of showing male copulatory behaviour. Similarly, no differences were obtained in the quality or quantity of lordotic behaviour displayed on four weekly tests with a vigorous male. Ward 1974 concluded that "the prenatal stress treatment had little effect on the female fetuses" (p10).

Since this early work, a variety of female behaviours has been investigated, ranging from preconception estrous and sexual receptivity, to parturition length and efficacy and postpartum maternal/progeny maintenance behaviours. As with several areas of research reviewed in this thesis, there are variations in results emerging from different laboratories, the lack of consistency

being best explained in terms of the methodologies employed by the various research groups (Beckhardt and Ward 1983). Typically, Herrenkohl and her co-workers (Herrenkohl and Politch 1978; Herrenkohl 1979a; 1979b; Herrenkohl and Gala 1979; Politch and Herrenkohl 1979; 1984) using a severe stressor have reported significant effects of prenatal stress, whereas Ward and her colleagues have not (Ward 1974; Meisel 1980; Beckhardt and Ward 1983).

Considering first preconception [16] effects, compared with controls, prenatally stressed females have been found to exhibit later vaginal opening (Politch and Herrenkohl 1984), to have longer estrous cycles (Herrenkohl and Politch 1978; Herrenkohl 1979a; Herrenkohl and Gala 1979; Burack 1982; Politch and Herrenkohl 1984; Herrenkohl and Scott 1984) and metestrus cycles (Herrenkohl and Politch 1978) and higher median quality receptivity scores (Burack 1982; Politch and Herrenkohl 1984).

However, there have also been some non significant results, for example Chapman, Masterpasqua and Lore (1976) found that prenatal crowding did not influence fertility scores, whilst Allen and Haggett (1977) reported no differences between crowded ovariectomised testosterone-injected female offspring and controls, with respect to amounts of male copulatory behaviour. They did, however, report that crowding resulted in less sexually receptive females, a finding which contrasts with Politch and Herrenkohl's (1984) work. Furthermore, both Dahlof, Hard and Larsson (1977) and Rykaszewski (1985) have found no differences in estrous cycles in either prenatally crowded or restrained groups, a finding supported by Beckhardt and Ward (1983), who reported estrous cycles to be normal in their prenatally stressed animals. These results are divergent to Herrenkohl's and may well reflect the fact that her stressing procedures are particularily severe. As Beckhardt and Ward (1983) point out "it is possible that there is a sex difference in the amount of prenatal stress required to disrupt adult reproductive functioning. A more intense stressor may be required to modify the behaviour and physiology of females than is sufficient to alter the behaviour of males" (p117).

[16] Preconception effects in this instance refer to effects in prenatally stressed offspring that occur before they are mated and become pregnant and not to effects caused by manipulation of their mothers prior to their pregnancies.

Recently, there has been some evidence to suggest that fertility and fecundity are in some way dependant on prolactin surges occuring postcoitally (Kinsley, Mann and Bridges 1988b), whilst on a more general level, evidence is being amassed that the effects of prenatal stress on female offspring may be due to alterations in a number of endocrine, neuroendocrine and neurochemical systems (Fride, Dan, Gavish and Weinstock 1985; Kinsley and Bridges 1987; Moyer, Herrenkohl and Jacobowitz 1978; Kinsley, Mann and Bridges 1988a; 1988b). These underlying physiological correlates will be discussed in the next section, but are included briefly here to highlight the direction of current research; namely having found evidence of poor maternal behaviour in prenatally stressed females, research is now turning to biochemical levels of analysis for explanation.

With respect to the partum period, Beckhardt and Ward (1983) have reported no difference in gestation length between their prenatally stressed and control groups, whereas Herrenkohl (1979a) has not only reported stressed animals to have prolonged gestation periods when compared with controls, but also that approximately three times the percentage of prenatally stressed females failed to maintain pregnancy, when compared with controls. Additionally, twice the percentage of her prenatally stressed animals failed to become pregnant and irregularities including pseudopregnancies were observed in this group. Furthermore prenatally stressed females exhibited a higher instance of vaginal haemmorhaging during the first trimester and spontaneous abortions in the third trimester. Overall, twice as many control animals as stressed animals gave birth. In a second study, Herrenkohl and Gala (1979) reported that prenatally stressed females fell into two groups, those that did and those that did not maintain young. Interestingly, in the latter group, autopsy revealed that they had twice as many uterine implantation sites than number of fetuses born and that their serum prolactin levels had fallen significantly below both those who had maintained young and the control group. This, the authors felt, indicated that the prenatal stress consequences are "all-or-none: females either can or cannot breed and maintain young successfully" (p703). This may well explain the discrepancy between their findings and those of Beckhardt and Ward (1983). The latter authors may well employ a stressor which encourages all their females to breed successfully.

Finally, postpartum effects have also been reported. In particular, prenatally stressed animals are more prone to losing litters through stillbirths or neonatal mortalities and successful survival of young to 10 days postpartum is halved (Herrenkohl 1979). Furthermore, both Herrenkohl and Gala (1979) and Politch and Herrenkohl (1979) have reported that prenatal stress reduces offspring [17] littersizes and litterweights, although other researchers have not replicated these effects (Beckhardt and Ward 1983; Fride, Dan, Gavish and Weinstock 1985; Kinsley and Svare 1987). No significant differences in nursing behaviours have been reported, although Herrenkohl (1979a) has found low incidences of lactation amongst prenatally stressed animals, with only 26 to 40 percent of their offspring receiving milk compared to almost 100 percent of the offspring of the non stressed group. However, as before, this finding may well reflect the nature of the prenatal stressor, as Beckhardt and Ward (1983) have reported no differences in amounts of milk received between offspring of stressed and control animals.

Maternal behaviours, in particular crouching and retrieval as well as latency to retrieve have also been examined. Generally, if there is an effect of prenatal stress it is deleterious, female offspring of prenatally stressed mothers taking significantly longer to exhibit maternal behaviours than their non-stressed counterparts (Kinsley and Bridges 1986; 1988). However, it should be pointed out that not all studies have found differences between their groups (Beckhardt and Ward 1983; Fride, Dan, Gavish and Weinstock 1985).

Other than caretaking behaviours, protection of offspring has also been explored. Again, prenatal stress seems to have a negative effect. For example, when placed in a conflict situation, control animals were found to retrieve their pups twice as often as stressed animals (Fride et al 1985). Aggressive behaviours and infanticide are also affected by prenatal stress. For example, Miley, Blustein and Kennedy (1982) found that exogenous early postnatal testosterone combined with prenatal handling produced both more frequent and a more rapid rat-pup killing in female Long Evans rats. However, this behaviour appears to be genotypically regulated (Miley 1983). Number of attacks, lunges and bites are also modulated by genotype (Kinsley and Svare 1987), prenatal

[17] These are the grandpups of the females stressed during their gestation.

stress significantly increasing postpartum aggression in C57BL/6J female mice, but reducing it in DBA/2J animals. Maternal aggression is also lowered in CD1 mice (Politch and Herrenkohl 1979).

To date, there is no definitive explanation of why prenatal stress appears to reduce maternal behaviours in female animals. What does appear to be true, is that prenatally stressed females resemble normal males in their responses to young. Perhaps, as Herrenkohl and Scott (1984) have suggested the prenatally stressed female rat is exposed to higher than normal levels of androgen because of stress effects on ovarian cyclicity, masculinising her behaviour. Indeed, prenatal stress has been found to affect female endocrine responsiveness (Kinsley and Bridges 1987) and in particular those hormones that are mediated by the Medial Preoptic Area (MPOA) which also regulates expression of maternal behaviour (Numan 1983). It may be that prenatal stress is having an effect on this region whilst it is developing, although the exact mechanisms are still to be elucidated.

To summarise, the more severe types of prenatal stress, such as those employed by Herrenkohl and her colleagues produce clear effects in most types of female sexual and maternal behaviours. Milder stressors, however, either have no effects, or are mediated by the animals' strain. As yet no explanation for these results is apparent in the literature, although increasingly it appears that the effects are being mediated at neurological level.

Prenatal stress has been shown to influence the maturation of behaviour. As with previous findings the nature of the stressor is important. Sobrian (1976) who employed prenatal handling, found no differences between her stressed and control groups, in either a free-fall righting reflex, or in the auditory startle response. With the more severe stressor, restraint, however, Barlow, Knight and Sullivan (1978) reported that prenatal stress significantly retarded the appearance of these dynamic postural adjustments as well as detrimentally influencing the cliff avoidance response. The results of the latter authors' work suggest that "maternal restraint does have significant adverse effects on the postnatal growth and development of the offspring" (p216), but

"the mechanism by which maternal restraint stress affects the developing fetus in the prenatal period to cause postnatal retardation is not known" (p217). Barlow et al (1978) also investigated the development of more complex skills and orienting responses, including choice of jumping down to an empty cage or the home cage from a platform, ability to cross a narrow path to reach the home cage and length of time animals could maintain position on a rotating circular wooden block. With respect to the jumping response, animals stressed on days 18-20 of gestation would not leave the platform, whereas animals either stressed during days 9-11, 12-14, 15-17 or from control mothers did jump down. No differences emerged between the groups on the other two behavioural tests. The authors have interpreted these results as reflecting an impairment to orient to the home cage in the stressed group and furthermore, have found that these effects were most marked in stressed pups reared by stressed mothers and least marked in controls reared by controls, with the other two crossfostered groups being intermediate. This indicates that the effects were induced partly prenatally, at the time of treatment and partly postnatally by rearing by mothers that had been stressed.

Fride and Weinstock (1984) have also investigated behavioural maturation, in particular concentrating on motor development (righting reflex, cliff avoidance, turning on an inclined plane and swimming behaviour) and the development of a motivation-involved behaviour (home seeking test). In their experiments, pregnant rats were exposed to different schedules of noise and light stress and the timing of these schedules provided an important variable in altering the nature of the offsprings' responses. Thrice-weekly *random* stress resulted in a delay in the development of all behaviours studied, whereas *daily* stress exposure throughout pregnancy resulted in normal behavioural and physical development. Rats exposed to *daily* stress in the last week of pregnancy, however, produced litters that displayed accelerated development of all parameters leading these authors to conclude that it is "the unpredictable nature of prenatal stress (that) is responsible for delays in behaviour of offspring" (p651). In addition and related to maturation, Ohkawa (1987) has investigated play behaviour and has found that forced immobilisation of the mother during her gestation has differential effects on male and female offspring. In particular, prenatal

stress causes a significant depletion in social play in males, but a significant increase in play amongst females. Okhawa has suggested that this result reflects the effects of prenatal stress on the sexually dimorphic differentiation of male and female brains, but as yet positive proof is lacking.

So, both the nature and predictability of the stressor are important factors in the development of behaviour. In some instances, prenatal stress can have an ameliorating effect, in others, a detrimental one. This is of particular interest, as in most cases reviewed in this section, stress has been considered as having a negative effect on offspring. The finding that predictable stress, late on in pregnancy can be efficacious, is rather unusual in this literature.

Offspring Physiology

As well as behavioural maturation, physiological development has also been investigated, the most common measure taken being that of body weight. As with the behavioural data outlined above, the nature of the stressor has a profound effect on this measure. Furthermore, the age at which the animals' weights are taken also affects the results. Prior to weaning, physical stressors such as handling and restraining the mother have a detrimental effect on offspring body weights when compared with control animals (Werboff, Anderson and Haggett 1968; Herrenkohl and Whitney 1976; Barlow, Knight and Sullivan 1978; Chapman and Stern 1978; Herrenkohl 1979a; Politch and Herrenkohl 1979; Burack 1982; Anderson, Rhees and Fleming 1985; Ward and Reed 1985; Kinsley and Svare 1986b; Lephart, Fleming and Rhees 1989 [18]), whereas crowding the mother (Allen and Haggett 1977; Dahlof, Hard and Larsson 1977; Moore and Power 1986; Power and Moore 1986; Peters 1988a; Peters 1988b; Peters 1990) or exposing her to light and noise (Fride and Weinstock 1984; Fride, Soreq and Weinstock 1986; Fride and Weinstock 1989) during gestation have little or no effect on offspring weights [19]. In adulthood, however, weight

[18] The only study to employ restraint and find no significant differences between stressed and non-stressed groups' birth weights is that of Weinberg (1987), the exception to prove the rule.

[19] Unfortunately, the picture is complicated by Plaut, Grota, Ader and Graham's (1970) finding that pups of handled mothers were *heavier* than their control counterparts, however, in this instance there were fewer stressed pups per litter than control pups, which may have accounted for this deviation from the norm.

differences tend to disappear, most studies reporting no significant differences between their prenatally stressed and control offspring (Ader and Conklin 1963; Plaut, Graham and Leiner 1972; Ward 1976; Allen and Haggett 1977; Moyer, Herrenkohl and Jacobowitz 1978; Meisel, Dohanich and Ward 1979; Chapman and Stern 1979; Fride and Weinstock 1984; Kinsley and Bridges 1987; Weinberg 1987; Peters 1988a; Peters 1988b; Kinsley, Mann and Bridges 1988a; Fride and Weinstock 1989). Indeed, in the literature, only one study (Dahlof, Hard and Larsson 1977) has reported any weight differences between adult offspring, in this case males of mothers crowded during gestation being heavier than controls at 115 days of age.

As well as offspring weights, litter sizes and sex ratios have also been measured. Predominantly, no significant differences have emerged between groups in either measure (Allen and Haggett 1977; Dahlof and Larsson 1977; 1978; Chapman and Stern 1978; 1979; Wilke, Tseu, Rhees and Fleming 1982; Anderson, Rhees and Fleming 1985; Kinsley and Svare 1986b; Fride, Soreq and Weinstock 1986; Moore and Power 1986; Power and Moore 1986; McLeod and Brown 1988) although there have been a few exceptions. Specifically, Werboff, Anderson and Haggett (1968) found that handled mice had larger litters than controls, whereas Chapman, Masterpasqua and Lore (1976) found that prenatal crowding reduced littersizes and both Chapman and Stern (1978) and Herrenkohl (1979a) have reported than prenatal restraint has a similar effect. Furthermore, Burack (1982) has reported that prenatally stressed mothers produce fewer live pups, whilst Plaut, Grota, Ader and Graham (1968) have reported differences in sex ratios, there being more male offspring in their control group than in their prenatally handled group. This latter finding is particularily interesting, as it has been suggested that the male zygote and fetus are less likely than their more viable female counterparts, to either implant or develop, when the mother is under stress. It is known that there are more male than female losses in all stages of prenatal development (National Centre for Health Statistics 1967) and maternal stress, in inhibiting male development would affect sex ratios. In a study specifically designed to investigate this phenomenon (Lane and Hyde 1973), severely stressing mothers by placing them in individual wire cocoons which totally restricted movement had significant effects on both sex ratios and

number of offspring. Stressed rats produced significantly more females than males and overall, significantly fewer offspring than unstressed controls.

Other measures of physiological maturation have included ear opening, eruption of incisors and eye opening (Barlow, Knight and Sullivan 1978), with prenatally stressed offspring typically demonstrating retarded maturation. Additionally, anogenital distance has also been calculated, prenatally stressed animals typically having a shorter A-G distance than controls, (Dahlof, Hard and Larsson 1978; Wilke, Tseu, Rhees and Fleming 1982; Burack 1982; Moore and Power 1986; Power and Moore 1986; Lephart, Fleming and Rhees 1989 [20]) although there have been reports of no significant differences in A-G distance by some researchers (Ward 1976; Chapman and Stern 1978), Ward (1976) also failing to find any differences in penile length or spermatogenesis in her prenatally stressed and control groups.

As well as general body measurements, gland and brain weights have also been calculated. As part of the research in this area has concentrated on male sexual behaviour following castration, a by-product has often been the inclusion of testes weights in the results section. When this has occured, quite a few studies have reported no significant differences between prenatally stressed and control groups (Ward 1976; Chapman and Stern 1978; Politch and Herrenkohl 1984; Lephart, Fleming and Rhees 1989). However, studies have found differences, Dahlof, Hard and Larsson (1978), Chapman and Stern (1979), Pollard and Dyer (1985) and Kinsley and Bridges (1987) in favour of their restrained offspring and Meisel, Dohanich and Ward (1979), Burack (1982) and McLeod and Brown (1988) in favour of their control animals. Burack (1982) has also noted delayed testes descent in prenatally stressed animals. One study has investigated testes' weights within 12 hours of birth (Chapman and Stern 1978) reporting no significant differences between groups, however, during gestation, fetal testicular weight differences have been observed, stressed offspring having smaller testes (Wilke, Tseu, Rhees and Fleming 1982). As well as testes, prenatal stress has also been found to alter ovarian weight (Herrenkohl and Scott

[20] This last study did not quite achieve statistical significance although stressed animals were below control values.

1984), offspring of prenatally stressed dams having heavier ovaries that offspring of non-stressed dams. Interestingly, uterine weights of the stressed group were lower than their non-stressed counterparts, a factor which might explain the higher incidence of stillbirths amongst stressed animals reported by Herrenkohl in 1979.

With respect to adrenal weights, Dahlof, Hard and Larsson (1977; 1978) have reported their prenatally stressed offspring to have heavier adrenals, a finding confirmed by Herrenkohl and Scott (1984) whilst Chapman and Stern (1978; 1979) have reported the opposite. Fetal adrenal glands have also been investigated, weight differences emerging in favour of control animals by day 20 of gestation (Wilke et al 1982) but no differences being reported between the groups when adrenal weights were measured at 18 days of gestation (Lephart et al 1989).

One final piece of anatomical investigation which certainly deserves some attention concerns the effects of prenatal stress on the brain. The earliest report concentrated on brain weight (Plaut, Graham and Leiner 1972) and found no differences between the groups, although some perinatal experiential effects emerged reminiscent of the enrichment literature. This lack of brain weight difference between the groups has since been confirmed (Peters 1990). Brain regions which are thought to be sexually dimorphic have also been investigated, with some interesting results. The first evidence that prenatal stress may alter morphological development in the central nervous system was provided by Whitney and Herrenkohl (1977). According to Herrenkohl (1983) "the then current belief on the neural basis of sexual behaviour was that masculine sexual behaviour appeared to be mediated by a system involving the pre-optic nucleus and medial forebrain bundle and that feminine sexual behaviour involved the anterior hypothalamus, habenula and medial central hypothalamus" (p177). Reasoning that lesions of the anterior hypothalamus would interrupt feminine but not masculine sexual behaviour, Whitney and Herrenkohl (1977) operated on prenatally stressed male animals (who demonstrate feminine sexual behaviour) and found a decrease in lordotic responses in these animals. Sham-operated prenatally stressed males, however, continued to produce lordotic responses.

Since this early work, Anderson, Rhees and Fleming (1985) have also examined the effects of prenatal stress on the development of the sexually dimorphic nucleus of the preoptic area (SDN-POA) and have found that prenatal stress reduces the SDN-POA of male offspring by 50% when compared with the nuclear areas of control males. The size of the SDN-POA of female offspring, however, was not significantly altered by prenatal treatments. In a further paper, (Fleming, Anderson, Rhees, Kinghorn and Bakaitis 1986), sexually dimorphic asymmetries in the cerebral cortex were examined, with similar results, namely that prenatal stress affected male offspring but not female offspring. In this instance, normal male anatomy was biased in the direction of a feminine structure, a finding which is consistent with the demasculinised and feminised behaviour patterns in prenatally stressed males, first reported by Ward (1972).

Hormonal and neurochemical assays have also been carried out on prenatally stressed animals, emphasis being placed on those biochemicals that are thought to relate to stress and sexual dimorphication. Considering first the hormones, several authors have investigated plasma corticosterone levels (Ader and Plaut 1968; Ader and Deitchman 1970; Politch, Herrenkohl and Gala 1978; Peters 1982; Ward and Weisz 1984; Rykaszewski 1985; Fride, Dan, Feldon, Halevy and Weinstock 1986; DeTurck and Pohorecky 1987; Weinberg 1987; Pohorecky et al 1989), as well as serum prolactin levels (Politch, Herrenkohl and Gala 1978; Herrenkohl and Gala 1979; Kinsley, Mann and Bridges 1989), estradiol-induced (Kinsley and Bridges 1987) or stress-induced prolactin release (Kinsley, Mann and Bridges 1989) testosterone levels (Ward and Weisz 1980; 1985; Anderson et al 1985; 1986) androstendione levels (Wilke, Tseu, Rhees and Fleming 1982) and progesterone levels (Ward and Weisz 1984). Results are difficult to interpret, but what is clear is that prenatally stressed and non stressed male and female animals have differential patterns of responding.

Looking at the sex hormones first, as adults, prenatally stressed male rats have been found to have lower circulating levels of testosterone relative to their non-stressed peers (Anderson et al 1985; 1986). Prenatal stress has also been found to alter testosterone titers in male fetuses, changing

both the absolute levels and temporal patterns. Compared to controls, plasma testosterone concentrations in prenatally stressed male rats have been found to be higher on day 17 (in utero) and lower on day 18, decreasing in stressed males at a time when they were increasing in the controls (Ward and Weisz 1980; 1984). This testosterone surge occuring prematurely in prenatally stressed rats, has led Ward and Weisz (1980) to speculate that the "prenatal stress syndrome", characterised by impaired male copulatory behaviour and an enhanced female lordotic potential, could result from a desynchronisation between central nervous system maturation and patterns of testosterone secretion by the testes during the latter stages of fetal growth. This hypothesis is based on the generally accepted view that in the male rat, testosterone produced by the Leydig cells during perinatal development is necessary for the normal differentiation of those neuronal mechanisms that regulate sexual behaviour in the adult (Orth et al 1983). In normal male fetuses, there is also a close correlation between the pattern of circulating testosterone and the activity of a key steroidogenic enzyme $\triangle^5$-3β-hydroxysteroid dehydrogenase (3β-HSD) in Leydig cells (Orth and Weisz 1980) and as Orth et al (1983) have reported, in prenatally stressed fetuses, patterns of this enzyme are altered in a manner which parallels prenatal effects on testosterone. In particular, peak activity of this enzyme, which occurs in normal animals around days 18 and 19 of gestation, is lacking in prenatally stressed fetuses. However, Orth et al (1983) did report higher rates of activity of 3β-HSD on gestation days 16, 17, 20 and 21, that is peak surges occuring earlier and later in prenatally stressed animals than in controls. This has led these authors to suggest that "the abnormal ontogenic pattern of circulating testoseterone in stressed fetuses results from a disruption of normal steroidogenic function of Leydig cells rather than from alterations in the clearance rate of testosterone from the circulation" (p 628). Furthermore, although the mechanisms by which prenatal stress alters the developmental pattern of 3β-HSD is unknown, Orth et al (1983) have suggested that as testicular steroidogenic activity can be regulated by luteinising hormones (LH) or prolactin, then the early peak of 3β-HSD before day 18 and 19 of gestation may be due to the premature release of LH from the fetal pituitary. That is, prenatal stress effects are being mediated via the fetal pituitary, itself under CNS control.

Androstenedione, a product of both adrenal and testicular steroid synthesis in the rat, is also capable of altering offspring sexual development. Wilke, Tseu, Rhees and Fleming (1982) have investigated whether prenatal stress has any effect on this hormone and have found that exposing female rats to restraint, heat and light elevated fetal androstenedione levels, a finding which they believe may be, in conjunction with the fetal testosterone patterns, a basis for altered sexual behaviour in prenatally stressed animals.

Androstenedione and testosterone are not the only sexual hormones to have been investigated, however. The effects of prenatal stress on prolactin levels and progesterone have also been reported. With serum prolactin, levels have been found to be markedly reduced in prenatally-stressed females postpartum (Herrenkohl and Gala 1979) and in both males and females following *Ether* stress (Politch, Herrenkohl and Gala 1978) or *Estradiol* (Kinsley and Bridges 1987) or *restraint* in adulthood (Kinsley, Mann and Bridges 1989). Baseline levels, however, are unaffected by prenatal experience. Typically, prenatally stressed females have higher levels of prolactin than their male counterparts (Kinsley et al 1989), which these authors attribute to differential patterns in the CNS of male and female prenatally stressed rats. With progesterone, however, in both stressed and control groups and in male and female animals, concentrations have been found to be identical, following a pattern of decline between days 19 and 21 of gestation (Ward and Weisz 1984).

Corticosterone, one of the main hormones implicated in stress, has also received attention. For example, it has been reported that offspring of animals handled throughout pregnancy show adrenocortical activity rhythms as early as 18 days postpartum, unmanipulated controls not displaying rhythmicity until they are 25 days old (Ader and Deitchman 1970). However, this finding is complicated by the fact that basal and stress induced corticosterone levels show sex, age and strain differences. For example, Peters (1982) found greater elevations in plasma corticosterone levels in 23 day old prenatally stressed Sprague-Dawley offspring in a reaction to stress test, whilst Politch, Herrenkohl and Gala (1978) reported no differences between their stressed and

non stressed Sprague-Dawley rats when samples were taken at 160 days. Rykaszewski (1985), on the other hand, observed that prenatal stress decreased both basal and stress-induced concentrations in female offspring of a genetic line bred for poor shuttlebox avoidance conditioning (LA). With Sabra rats, baseline levels have been found to be unaffected by maternal experience, but after exposure to an open field corticosterone levels were significantly higher in the prenatally stressed animals (Fride et al 1986).

Prenatal stress has also been found to modify the organism's corticosterone reaction to some "recreational" drugs. In particular, offspring of Sprague-Dawley dams handled whilst pregnant had lower plasma corticosterone levels following ethanol injections (DeTurck and Pohorecky 1987) but did not differ from nonstressed controls following caffeine injections (Pohorecky et al 1989). Prenatal restraint stress has also been reported to alter sensitivity to ethanol (Weinberg 1987), prenatally stressed offspring in this group showing an increased adrenocortical response, when compared to nonstressed controls, although this latter finding did not attain statistical significance. Why one study should report an increase in corticosterone responsiveness, the other reporting the opposite, is at present unclear. It may well be that, as with many other studies in this area, the nature of the stressor plays an important role in mediating the physiological and/or behavioural response.

The effects of prenatal stress on neurochemicals have also been examined in the literature (Plaut et al 1972; Moyer et al 1977; 1978; DeTurck and Vogel 1972; Peters 1982; 1984; 1986a; 1986b; 1988a; 1988b; 1990; Fride et al 1985; Fride and Weinstock 1986; 1988; 1989). In one of the earliest studies in this area Plaut, Graham and Leiner (1972) found that prenatal handling decreased serotonin (5-HT) levels in 21 day-old offspring tested at the trough of the 24-hour serotonin rhythm. This is of particular interest, as Ferreira (1965), reviewing evidence for teratogenic effects of serotonin and its involvement in various abnormalities of pregnancy had suggested that serotonin might be one of the mechanisms of the influence of prenatal emotional factors on offspring development. In a later study (Peters 1982) reported that low level prenatal stress altered

the brain levels of serotonin and its major metabolite 5-hydroxyindoleacetic acid (5-HIAA). The 5-HT and/or 5-HIAA levels in cerebral cortex and pons medulla were significantly increased at 16 days, but unchanged at 23 and 60 days of age. These short-lasting increases in 5-HT and 5-HIAA may be consistent with the idea that "stress accelerates the onset of differentiation of nerve cells known to contain 5-HT terminals" (p724). In contrast, the hypothalamus showed a reduced 5-HT level at 16 days and an increased 5-HT level at 60 days of age which suggested that there may have been a long-lasting change in functioning of central 5-HT neurons. Maternal low-level stress has also been found to produce persistent changes in 5-HT receptor binding in several brain regions of the offspring and increased behavioural responses to 5-HT agonists (Peters 1986a; 1986b). This has been interpreted as providing "additional evidence that prenatal stress affects the development of serotonergic neurons and it is possible that such changes may underlie the reported behavioural deficits in offspring of stressed female rats" (Peters 1986b p1377).

Over the last few years, Peters has concentrated on examining the origins of these effects and in particular has found that the impact of crowding the pregnant dams and giving them once-daily saline injections throughout the gestation period is mediated both prenatally and postnatally via the mother-infant interaction (Peters 1988a). In this experiment, offspring were crossfostered such that offspring of stressed mothers were reared by control mothers and vice versa. Tested at 60 days of age on a behavioural response to to the 5-HT receptor agonist 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) which produces a characteristic syndrome consisting of hyperactivity, hyperpyrexia, head weaving, forepaw treading and hindlimb abduction, development of the offspring was found to have been affected by the presence of a previously stressed parent, that is mediated both pre and postnatally. Specifically, prenatally stressed progeny reared by control dams showed a reduced behavioural response to 5-MeODMT and increased 5HT2 receptor binding in the cerebral cortex, whilst control progeny reared by stressed mothers demonstrated an increased behavioural response to the agonist as well as increased 5HT2 receptor binding. Peters (1988a) suggests the 5-HT syndrome must be mediated by different mechanisms

pre and postnatally. "One mechanism, which operates in utero, produces a significantly reduced behavioural response to 5-HT receptor activation. A second mechanism, which appears to involve an early postnatal interaction between previously stressed mothers and the offspring results in a marked increase in the 5-HT syndrome" (p672). He then continues by questioning the possible nature of the the mechanism which mediates the effects of prenatal stress on serotonergic neurons, suggesting that "one possibility is that glucocorticoids may be involved" (p672). The rationale for this proposal is that high levels of glucocorticoids both delay cellular maturation (for example synaptic development in the CNS) and are found in the maternal circulation.

In an attempt to further define the critical prenatal period for stress-induced changes in the development of the serotonergic neurons, in a second experiment (Peters 1988b) stress treatments were administered at different gestational periods. Stress-induced changes in the intensity of 5-HT mediated behaviours were found to occur during the final seven days of pregnancy a time when 5-HT containing cells first make their appearance. Given that serotonin is believed to play a role in early brain development (Lauder and Krebs 1978), Peters (1990) has suggested that "an increase in fetal brain 5-HT synthesis may be involved as a mediator in at least some of the effects of prenatal stress" (p943).

Serotonin is not the only neurotransmitter to come under observation. Moyer, Herrenkohl and Jacobowitz (1978), for example, have investigated the effects of stress during pregnancy on the catecholamines norepinephrine (NE) and dopamine (DA), in discrete brain regions of the offspring as adults. Their rationalisation for investigating these neurotransmitters was simple, namely that there is evidence that brain catecholamines are involved in the expression of some sexually differentiated responses (Ahlenius, Engel, Eriksson, Modigh and Sodersten 1975; Soulairac and Soulairac 1975). As expected, differences were found and in regions associated with reproductive processes including the medial preoptic nucleus and median eminence. Interestingly, prenatal stress also affected discrete brain regions of female offspring, in such a way as to alter prolactin secretion, thus influencing reproductive functions that are prolactin-related such as pregnancy,

lactation or maternal behaviour. This work corresponds with the findings of reduced serum prolactin levels in prenatally stressed females postpartum, mentioned earlier. Since this early work on catecholamines, Peters (1982) and Fride, Dan, Gavish and Weinstock (1985) and Fride and Weinstock (1986) have also reported changes in NE and DA levels in offspring of prenatally stressed animals. Furthermore, Fride et al (1985) have also reported that the number of benzodiazepine receptors in the hippocampi of prenatally stressed females was significantly lower than in the controls, a finding that they interpret as increasing their vulnerability to stressful situations, whilst Peters (1984) has noted that prenatal stress delays the development of noradrenergic neurons.

More recently, prenatal stress has been found to alter cerebral lateralisation of dopamine activity (Fride and Weinstock 1988) producing effects which last into adulthood (Fride and Weinstock 1989). In addition, the effect on cerebral lateralisation is more evident in females than in males, perhaps explaining the sex differences that are increasingly common in the prenatal stress literature. Interestingly, testosterone, which has already been implicated as a possible mediator of the prenatal stress effects (Ward and Weisz 1980; 1984) has also been found to be involved in the development of cerebral lateralisation (Geschwind and Galaburda 1985). Speculation concerning the role of this hormone in the development of the prenatal effects is still continuing.

Finally, as the "effects of prenatal stress have many features in common with the behavioural and physiological actions of opiates and endogenous opoids [21]" (Kinsley at al 1988a p123), a new line of research has started to investigate the role of prenatal stress in the development of opioid systems (Kinsley et al 1988a; Insel et al 1990). As with cerebral lateralisation, a common feature of the ontogeny of the μ type opioid receptor system is that it can be altered by testosterone. Kinsley at al (1988a) therefore investigated the effects of prenatal stress (heat and restraint) on morphine and stress-induced analgesia. Results demonstrated that prenatally stressed females displayed lower pain thresholds than controls following cold water swimming, whislt no differences were found between the stressed and unstressed males. With morphine-

[21] This system modulates a variety of sexual, maternal and aggressive behaviours.

induced analgesia, stressed females displayed greater analgesia than controls, the pattern of responding being the opposite in the male groups. Possible mechanisms for these effects of prenatal stress on opiate sensitivity, according to the authors could involve "insensitivity to gonadal steroids" (p127) as it has been reported (Chatterjee et al 1982) that "the presence of testosterone potentiates the effects of morphine on analgesia" (p127).

In a follow-up study, given that there is this sensitivity to morphine in prenatally stressed animals, Insel et al (1990) examined the effects of heat and restraint stress on μ opiate receptor numbers. Offspring or stressed dams exhibited reduced receptor numbers in caudate-putamen and nucleus accumbens, lateral amygdala and the endopiriform nucleus. Interestingly, no sex differences emerged, especially given the sexually dimorphic opiate sensitivity noted in the previous study (Kinsley et al 1989a). This may be because opiate receptors in the spinal cord and brainstem (which might be involved in pain reception) were not included in this assay. Several hypotheses were advanced by Insel et al (1990) to explain their results including "stress-induced increases in endogenous opiates could lead to a homologous down-regulation of these abundant receptors in the fetal brain" (p96) or that "opiate receptors are not decreased per se, but that either more cells or more processes with opiate receptors are eliminated in the prenatally stressed pups" (p96). As yet, causality is not established, but what is of relevance to the present discussion is that prenatal stressors have a lasting impact on brain opiate receptors, furthering our understanding of the consequences of manipulation of the maternal generation.

In summary, therefore, prenatal physical stressors have a variety of effects on offspring behaviour, physical maturation, anatomy, endocrinology and chemistry. As with the psychological stressors outlined in the previous section, the effects are complex and depend on both the timing and nature of the stressor, as well as the sex, strain and age of the offspring. To date, there are no definitive explanations of the mediation of these effects, although increasingly the causes appear to lie in the hormonal mediation of neuroanatomical and neurochemical development.

c) Pain Stressors

The literature on the effects of prenatal stress is fraught with methodological and conceptual problems most of which have been dealt with earlier in this section. One factor which warrants more discussion, however, concerns the quality and quantity of the prenatal stressor. This section could include those few studies that have employed punitive stressors such as audiogenic seizure (Thompson and Sontag 1956), uncontrollable tail shock (Takahashi et al 1988; 1990) or footshock either in a conflict situation (Joffe 1965c) or in a random presentation sequence (Sobrian 1976; 1977; Joffe 1977; Becker and Kowall 1977; Pereira, Ardila and Figueroa 1980). However, these procedures do not conform to the criteria set out in the introduction to this chapter, namely, they do not have much in common with the manipulation employed in the present thesis. Furthermore, they do not affect the offspring *indirectly* through the maternal response, but are likely to have an effect by altering the offspring physiology *directly*. Indeed, with respect to audiogenic seizure, Thompson and Sontag (1956) have suggested that their finding that offspring of stressed animals were significantly slower in water-maze learning, may have reflected the effects of "fetal anoxia due to spasmodic contraction of the uterine arteries, maternal blood changes due to shock, maternal endocrinological changes and the effects of direct sound stimulation on the fetus" (p456). Thus it appears that these effects are not mediated indirectly and consequently results from this type of procedure will not be included in the present review.

With respect to both tail and footshock [22], however, inescapable shock appears to have a psychological component (Weiss 1968), and as such deserves inclusion as a maternal psychological stressor. At any rate, as Becker and Kowall (1977) have remarked, "what is being studied is the effect of the manipulation on behaviour and a breakdown of the intervening sources of the effect can be attempted after the effect has first been established" (p1433).

As with the previous sections, a variety of physiological and behavioural effects of prenatal

[22] Joffe (1965c) has also pointed out that "it is unlikely that any direct stimulation of foetuses could have occurred" (p8) as the shock level employed are typically low (less than 3mA). The relative lack of severity of the stressor "also makes unlikely the possibility of foetal anoxia due to contraction of the uterine arteries "(p8).

pain stressors have been investigated. Considering the physiological effects first, few significant differences between offspring of stressed and unstressed groups have emerged. For example, Sobrian (1976; 1977) has examined the development of reflex actions and physical maturation (appearance of incisors, eyelid dysjunction, startle response, free-fall righting response) and has concluded that prenatal stress produces no generalised alteration in behavioural and physical ontogeny. Furthermore, in neither of her studies, both of which employed electric footshock in either the first or last trimester of pregnancy, did she find any changes in endogenous levels of 5-HT, 5-HIAA or NE. Both stimulated and control offspring exhibited the expected increase in steady-state levels of the amines as a function of age at 1, 12 and 25 days of age. However, some suggestion exists that the effects of prenatal stress on brain chemistry are triggered by weaning (Petropoulos, Lau and Liao 1972) and may therefore only be apparent in animals after this time. Alternatively, as Sobrian (1977) points out "NE, 5-HT and 5-HIAA levels... may essentially be uncorrelated with behavioural effects" (p49).

Takahashi et al (1988; 1990) have also considered the impact of a painful prenatal stressor on offspring physiology and have reported that offspring of rats exposed to uncontrollable electric shock during pregnancy exhibited significantly higher plasma ACTH and corticosterone concentrations than offspring of either mothers that experienced controllable shock or no manipulation at all (Takahashi et al 1988). So, the notion of controllability of the stressor is an important factor in the mediation of prenatal effects, and highlights the importance of hormones in the prenatal stress syndrome. In a second study, Takahashi et al (1990) considered the hormonal responses of these offspring further, looking at stress-induced secretion of ACTH at 14 days old and 21 days old. Plasma ACTH was significantly elevated in prenatally stressed animals (stress in this experiment consisted of uncontrollable tail shock) when only 14 days old, but lower than control males at 21 days.

These changes in the level of plasma ACTH with maturation are particularly interesting, as both the hypothalamic-pituitary-adrenal system and opioid system have been implicated in ACTH

secretion (Guillemin and Rosenberg 1955; Buckingham 1982; Siegel et al 1982) and may therefore be the mechanisms that mediate the effects of prenatal stress. However, as pointed out in the preceding section, the role of opioids in the regulation of prenatal stress effects have only recently attracted attention and as yet, no firm conclusions can be drawn from the data.

Takahashi et al (1990) also examined the effects of their tail shock stressor on parturition data and body weights. With respect to gestation length, no differences were found between the stressed and nonstressed groups, however, where stillbirths and fetal reabsorption did occur, it was always in the stressed group. Body weights of prenatally stressed and control offspring "that were tested" (p359) did not differ from each other.

Sobrian (1977) did, however, find weight differences in her groups. In particular her non stressed pups (C) were lighter than either early (EPS) or late (LPS) stressed animals at 6 days of age, but C animals were significantly heavier than EPS but not LPS at 30 days of age, demonstrating differential growth patterns. Similar results were reported by Pereira, Ardila and Figueroa (1980). Joffe (1965c) on the other hand failed to find any littersize or litterweight differences in his control and stressed groups.

Reactivity has also featured in this literature, the earliest work utilising the ubiquitous open field (Joffe 1965c). In this instance, no significant differences emerged between offspring on any of the traditional measures of emotionality, including defecation, urination or ambulation, when tested at 78-80 days of age. Sobrian (1976), however, tested her animals at 30 and 60 days and found that ambulation was altered by prenatal maternal stress, the direction of the change depending on the level of stress to which the female had been exposed. In addition, prenatal stress altered the adaption rates of ambulation, rearing and grooming behaviour. Moreover, Sobrian (1976) reported that overall activity levels during the neonatal period were lower in the offspring of stressed mothers, in comparison with controls, a finding that is comparable to that of a saline control group employed by Joffe (1977) in which prenatal stress reduced activity in 86-96 day old animals.

Probably one of the most comprehensive investigations of the effects of inescapable prenatal shock on offspring behaviour, however, was a study carried out by Becker and Kowall (1977) in which prenatally stressed and control male rats were tested over 20 trials in an open field. A T factor analysis was then performed on the set of observations and factor scores subjected to elevational analyses [23]. Results were complex, but pointed to the role of both prenatal and postnatal maternal environments in the expression of prenatal stress effects. In particular, male rats subjected to prenatal stress were found to acquire emotional reactivity levels in adulthood, that were either elevated or reduced depending on the postnatal maternal environment. Moreover, ambulation during both early and middle trials reflected two process dimensions which the authors labeled *emotional reactivity* and *characterisitic rate of habituation.* Interestingly, Becker and Kowall (1977) also discovered a second-order factor, which they suggested determined both habituability and exploratory behaviour, which was completely independant of emotional reactivity. Thus they hypothesised "the existence of two processes set in motion simultaneously the moment the rat is placed in the arena. One is to explore the terrain and one is to inhibit that exploration" (p1437). This work is not dissimilar to the two factor theory of Denenberg and his co-workers, outlined in chapter two. The only study to examine exploration per se (Pereira, Ardila and Figueroa 1980) reported significant differences between offspring of females subjected to electric shock on the eighth day of pregnancy and offspring of controls. Unfortunately, this was reported in an abstract, and no further detals were given.

Most recently, Takahashi et al (1990) have considered the frequency of ultrasonic vocalisations of prenatally stressed and control pups at both 14 days of age and 21 days of age. Reasoning that the occurence of vocalisations becomes less in a less stressful situation they examined ultrasonic emmissions as a reaction to isolation stress and footshock and tail flick latencies in response to the tail being placed in water. In the 14 day-old animals, prenatally stressed progeny made

[23] In the usual or R analysis a number of different responses or tests are correlated across a number of organisms observed on the same occasion, but in the T analysis by contrast, a number of different occasions are correlated across a number of organisms by using the same response measure. The factor analysis may result in the extraction of two or more T factors which can be viewed as summary sets of unconfounded cross-sectional cuts representing stages in a process unfolding or running its longitudinal course over the occasions provided (Becker and Kowall 1977).

significantly fewer ultrasonic vocalisations than their control counterparts although no differences emerged between the groups in latency to tail flick. By 21 days old, however, the vocalisation difference between the groups had disappeared, there still being no differences between the groups latency to tail flick. In considering the vocalisation measure, the authors state that "these results suggest that behavioural responses induced by stress are no longer potentiated in prenatally stressed pups" (p362). However, they also point out that vocalisation serves a different function at these two ages. In the younger animal vocalisations are thought to be emitted to attract the mother, whereas in the weanling animal, altogether more independent of its mother, vocalisations would be less common, if appearing at all.

Finally, the ontogeny of spontaneous motor activity has also been investigated (Sobrian 1976; 1977), with significant results. Prenatally stimulated offspring were found to be more active than controls on days 1-10, but less active in the third week postpartum. The age of peak activity, a developmental landmark, occured in prenatally stressed pups around ten days of age, with maximum activity not appearing in controls until the third week. This effect was independant of both the gender of the offspring and the timing of the gestational stress.

The only other behavioural measure to be investigated in this area of research, has been learning behaviour. Joffe (1965c) has reported that offspring of stressed females scored significantly more errors than control animals in a Hebb-Williams maze and hypothesised that too rapid a myelination in the foetuses of "emotionally aroused mothers offered the best tentative explanation for the occurence of differences in intelligence without accompanying differences in emotionality" (p10). In a later paper, however, no significant differences emerged between prenatally stressed and nonstressed offspring in an avoidance conditioning task (Joffe 1977).

In summary, therefore, the use of shock as a prenatal stressor, has comparatively few major effects on offspring behaviour. Unlike the previous section, where physical stressors had some effect on physical maturation and on neurochemistry, pain stressors do not appear to have any effects on physiological ontogeny or NE, 5-HT or 5-HIAA levels, although plasma ACTH and

corticosterone differences have been noted by one group of researchers (Takahashi 1988). The only physical differences to emerge, concern offspring growth patterns, weight differences varying in direction according to age. With respect to behaviour, control animals appear to be more reactive than stressed offspring, but this is qualified by the nature of the postnatal maternal environment. One study has found stressed animals to be less efficient in solving a Hebb-Williams maze, but this learning difference does not necessarily appear in other learning tasks.

3:3:3 Summary of Prenatal Influences

In this section the effects of exposing pregnant animals to a wide variety of different stressors have been reviewed. Probably the most succinct analysis of the findings, albeit not the most recent, has been provided by Ader and Plaut (1968) who remarked "that the effects of prenatal maternal stimulation on offspring result from a complex interaction of factors such as the strain and previous experience of the mothers, the type of stimulation and the period of gestation during which it is administered, fostering, sex and age of offspring and the type of dependant measurements that are made" (p278). This position has not been changed substantially by any of the more recent publications.

What is particularily relevant to the present thesis, however, is that environmental stressors acting on the mother have strong effects on the offspring, further supporting the methodology employed in this present work. In the following section, evidence from those studies that have manipulated the maternal environment postpartum and found changes in offspring behaviour will be reviewed, to complete the discription of the effects of "maternal influence" on offspring behaviour.

3:4 PERINATAL INLUENCES

As outlined in the introduction to this chapter, the present thesis is concerned with exposing females to differential environments prior to pregnancy, which may indirectly affect the foetus through changes induced in the mother herself. In the preceding sections, manipulations which fall within the category of maternal stressor and which occured either prior to conception, or during pregnancy were reviewed. From this, it has become increasingly obvious that the mother has a tremendous influence on her offspring. In this present section, the aim was two fold: firstly to review those studies in which the mother was exposed to an external influence postpartum and to detail any effects on her offspring, and secondly, to consider to what extent maternal influences are themselves mediated postnatally. To date, no studies have imposed a stressor on the mother following parturition and examined the impact on her offspring, although Smotherman and Bell (1980) have recommended this methodology as one way of evaluating the mediating effects of the mother on offspring behaviour [24]. Consequently, the second and related question will be addressed in this review section, namely, to what extent are maternal influences mediated postnatally, rather than in utero, or put more simply, does the perinatal behaviour of the mother affect her offspring.

Historically, within the human literature, interest in the role of the mother in shaping the future of her child(ren) can be traced to Classical times. However, as Denenberg (1972) points out, although "it is intuitively obvious that the mother plays a critically important role... it has been difficult to verify this empirically, to determine the extent of her influence, and to isolate the mechanism involved." (p399). One reason for this has been the psychoanalytic bias of a number of the early investigators, which "led them to assume the critical importance of the mother and to interpret their findings as proof of this assumption rather than attempting to test the validity of the assumption" (p399). Other reasons have included the failure to pay adequate attention to the need for appropriate controls, or to consider alternative hypotheses to account for the

[24] In particular, they state that "it would seem important, if not necessary to assess the role of changes in maternal caretaking activities in programs of investigation which entail direct or indirect manipulation of the maternal environment" (p202).

data. Despite these limitations, however, early workers in this field such as John Bowlby (1951) and Rene Spitz (1945; 1946a; 1946b; 1951) have had enormous influence, both in stimulating research and bringing about changes in child care, with their findings that adequate mothering was a prerequisite for normal development.

Within the animal literature, awareness of postnatal maternal influences has emerged from several sources. Firstly, from the research on infantile experience per se, when it became obvious that the mother was "a highly complex multidimensional stimulus who interacts with her offspring in a reciprocal fashion" (Denenberg 1972 p400). Secondly, from the "maternal deprivation" literature where animals have been removed from their mothers at an early age. Thirdly, from the "rat-aunt" studies of Denenberg and his co-workers, in which animals are reared with lactating or virgin females. With respect to the present thesis, however, a clearer understanding of the effects of postnatal mediation of environmentally induced maternal influences, has come from those studies where the maternal manipulation has occured prior to pregnancy, or prenatally and where the offspring have been either fostered or crossfostered. All of these areas will be detailed in the following pages, with particular emphasis being placed on the latter area of research.

Considering first research on infantile experience [25] and in particular the infantile experiences of handling and shock (Levine 1969a; 1975), it has become obvious that these treatments typically have prolonged and lasting effects on the animal's behavioural and physiological responses as an adult (Levine 1962; Denenberg and Zarrow 1971). One question which has generated a large literature concerns the way(s) in which these early experiences exert their effects. Several hypotheses have been proposed [26] (Russell 1971; Smotherman and Bell 1980), including firstly, the direct action hypothesis of Levine (1962), in which early experience outcomes are thought to result in part from the direct action of the stimulation impinging on the organism. A second theory, the cooling or hypothermia hypothesis (Schaeffer et al 1962), suggests that early stimulation

[25] For major reviews of this area, consult Beach and Jaynes 1954; Salama and Hunt 1964; Schaeffer 1968; Denenberg 1968; 1969a; 1969b; Levine 1969a; 1969b; Thompson and Grusec 1970; Russell 1971; Archer and Blackman 1971; Daly 1973; Lee and Williams 1974; Denenberg 1977; Wong and Wong 1978; Denenberg 1982.

[26] None of these hypotheses is mutually exclusive.

effects result from the incidental cooling that pups receive whilst out of the nest during treatment. Thirdly, Levine (1956) has suggested a stress hypothesis, in that all treatments employed to provide infantile stimulation are in some way noxious or stressful to the young animal and in this way serve to modify the physiological systems that mediate stress reactivity in adulthood. Finally, a fourth hypothesis has focussed on the importance of changes in the maternal environment (Richards 1966; Barnett and Burn 1967; Meier and Schutzman 1968; Bell, Nitschke, Gorry and Zachman 1971; Hudgens, Chilgren and Palardy 1972). This "maternal mediation" theory (Smotherman and Bell 1980) suggests that the reported effects of early stimulation result not only from the effects of the stimulation on the pups per se, but are also mediated by changes in the nature of the mother-infant interaction. These changes occur subsequent to the stimulation and return of the stimulated pup to the nest. This hypothesis has been both redefined and more precisely stated by Bell, Nitschke, Bell and Zachman (1974), Smotherman, Mendoza and Levine (1977) and Smotherman and Bell (1980) and is based on the premise that changes in mother-infant interactions have the capacity to influence the ontogeny of behaviour in the pup. Indeed, Villescas, Bell, Wright and Kufner (1977) have shown that prohibiting interaction between mother and pups after handling the pups, significantly alters the developmental outcomes typically produced by this treatment.

The most convincing demonstration of the maternal mediation hypothesis was that of Bell et al (1974), in which maternal behaviour and pup ultrasonic vocalisation were found to vary systematically as a function of the intensity of the stimulation the pups had received. In this study groups of litters were exposed to three minutes of handling (H), two minutes of 5-6^{o}c cooling (2C) or five minutes of 5-6^{o}c cooling (5C) for the first five days postpartum. The authors measured ultrasounds and maternal behaviours simultaneously and reported parallels between the rate and persistence of vocalisations and the effectiveness of mothering the pups received. Specifically, pups in the H group vocalised infrequently and elicited little maternal attention, whereas in the 2C group, pups initially vocalised at high rates triggering effective mothering. In the 5C condition, pups vocalised somewhat less initially, but vocalisations persisted across the recording

period. Pups in this condition, as with the H condition, received ineffective mothering. Bell et al (1974) have suggested that a maternal factor enters into the curvilinear relationship between the intensity of early stimulation and adult behaviour first proposed by Denenberg (1964). Since this early work, Bell et al's findings have been replicated and extended (Barnett and Walker 1974; Bell and Little 1978; Cross and LaBarba 1978), with evidence that maternal physiology is also affected by manipulation of the pups (Smotherman, Weiner, Mendoza and Levine 1977a; 1977b; Smotherman, Mendoza and Levine 1977; Brown, Smotherman and Levine 1977). Furthermore, whilst most studies have examined maternal behavioural and physiological changes that take place immediately after the pups are returned to the nest (Smotherman and Bell 1980), there also exists evidence demonstrating that changes in mother-infant interaction are long lasting (Lee and Williams 1974).

A second area of research which has contributed to an understanding of the importance of the mother in the normal development of her offspring is the maternal deprivation literature. Reasoning that one way to study the functions of a mother's influence on her offspring's behaviour is to rear the animal without its mother, several laboratories have embarked on extended programmes to investigate just this question. Probably the most famous work has emerged from Harry Harlow's laboratory based at the University of Wisconsin (Harlow 1958; 1960; 1968; Harlow and Harlow 1965; Harlow, Harlow and Hansen 1963; Griffin and Harlow 1966; Suomi and Harlow 1977), working with rhesus monkeys. In their experiments, rearing animals with surrogate cloth or wire mothers (Harlow and Zimmerman 1959) had profound effects on later sexual and maternal behaviours. Interestingly, up until puberty the surrogate animals appeared to be "normal" but after this time gross behavioural incompetences emerged. This finding that the detrimental effects of maternal deprivation may not express themselves until late in development, is of particular interest and according to Denenberg (1972), suggests that "it is dangerous to conclude that certain forms of infantile stimulation or early experience are without effect until the animals have been studied well into maturity" (p401).

More recently, work has extended to include the squirrel monkey (Mendoza, Smotherman, Miner, Kaplan and Levine 1978; Hennesey and Kaplan 1982) and macaques (Rosenblum and Alpert 1977). Probably the research most relevant to the present thesis however, are those few studies that have investigated the effects of maternal deprivation in *rats*. One problem with this type of research is that rats are immature at birth and must be handled frequently in order to be fed. Consequently the effects of maternal deprivation are difficult to separate from the effects of the extra handling stimulation. Despite this, several interesting points have emerged. With respect to learning, in a study of neonatal rats being reared in an incubator, Thoman, Wetzel and Levine (1968) found differential learning patterns between incubator and mother reared animals by three days of age. By three weeks, incubator animals showed some superiority over mother reared littermates in performance on the Hebb-Williams maze (Thoman and Arnold 1968a) and greater changes over trials in measures taken in the open field. These results have led the authors to conclude that "in contrast to previous research on mother deprivation, the effects cannot be interpreted as indicating poorer performance on the part of the mother deprived animals" (p221). Development of maternal behaviour in these "deprived" rats has also been investigated (Thoman and Arnold 1968b) with few long term effects emerging. However, in this latter study, offspring of incubator reared animals showed a higher mortality rate and significant delay in eyeopening, suggesting that deleterious effects may only express themselves in the next generation.

The third area of literature to be considered in this review is the work of Denenberg and his colleagues (review Denenberg 1970), which for the purpose of this thesis has been called the "rat-aunt" literature. What makes this work particularily interesting is the manner in which the maternal environment was manipulated. In brief, the procedure involved fostering new born mice to lactating rat mothers. The importance of this paradigm lies in the way it enabled the researchers to separate experimentally the usual confounding of genetic, prenatal and postnatal environments present in all naturalistic situations and in the great majority of laboratory settings. If the experimentally reared mice exhibited different behaviours from those of control animals reared by mouse mothers, it could be considered that such behaviours were significantly affected

by the nature of the postparturient maternal environment.

The first experiment in this series (Denenberg, Hudgens and Zarrow 1964) fostered C57BL/10J mice to lactating Purdue-Wistar rat mothers, control mice remaining with their natural mothers. Starting at 50 days, mice offspring were tested for four days in an open field and at least five weeks later in a fighting box. Offspring reared by rats showed both reduced fighting behaviour and activity. These findings have since been replicated (Denenberg, Hudgens and Zarrow 1966; Hudgens, Denenberg and Zarrow 1967; 1968) and have led to the conclusion that the role of the mother is an extremely powerful one which can markedly influence species-specific aggressive behaviours, as well as activity patterns. This effect was also reported in Rockland Swiss-Albino mice, whose plasma corticosteroid levels following exposure to a novel environment were also calculated (Denenberg, Rosenberg, Paschke, Hess, Zarrow and Levine 1968). Mice reared by rat mothers were found to have a lower adrenocortical response than either control raised mice, or control raised rats.

In their next series of experiments, the mediating mechanisms were considered. One factor which had to be eliminated was the possible effects of rat milk on the mouse progeny. To do this, an ingenious procedure involving using a rat aunt instead of a rat mother was devised. Mice were left with their natural mothers, but virgin rats, their maternal behaviours primed by being exposed to infant rats [27], were introduced into either a litter of four-day old mice or placed with a pregnant mouse (Denenberg, Rosenberg, Paschke and Zarrow 1969). Mice reared in the presence of a rat aunt had significantly lower adrenocortical resonses to novel stimuli and were less active in the open field. However, the rat aunt preparation was quantitatively weaker than the rat mother preparation. Having eliminated the possibility that corticosterone and activity changes were brought about by the nature of the mothers' milk, as Denenberg (1970) points out, there is "a very strong implication that the changes are mediated through the mother's or aunt's behaviour towards the young" (p86). One observation of the rat aunt behaviour had suggested they were less maternal than rat mothers, so in order to maximise the effect of the

[27] See also work by Stern and MacKinnon 1978 detailing the priming of virgin females.

aunt preparation, a final series of experiments were undertaken. One way to maximise maternal behaviour is to thelectomise the animal (surgically remove the nipples), mate her and remove her pups post partum. This should provide a non-lactating maternal animal. Using this technique, rat aunts were placed in a cage with a mouse mother and pups, (Rosenberg, Denenberg and Zarrow 1970) and results similar to those obtained with the rat mother were observed. This has led Denenberg (1970) to conclude that it is the behavioural interactions between the mother and offspring which radically alter both the physiology and behavour of the offspring.

So far in this section, three discrete areas of research have been outlined, all of which demonstrate conclusively that the mother has a tremendous effect on her offspring in the postpartum period. Of more interest to the present thesis, however, are the postnatally mediated effects of mothers who have themselves been manipulated. Evidence pertinent to this particular issue emerges from those studies in which females have been exposed to environmental stressors prior to giving birth and where the postnatal effects have been partitioned out using fostering or crossfostering techniques [28].

POSTNATALLY MEDIATED EFFECTS

Of the studies detailed in this chapter, in which the mother was subjected to some form of *Maternal* stressor [29], only 50 have employed any fostering or crossfostering techniques and of these, 24 studies have deliberately avoided any postnatal influences, by crossfostering experimental and control groups to naive females, thus standardising the postnatal environment (Joffe 1965b; Ader and Plaut 1968; Porter and Wehmer 1969; Ader and Deitchman 1970; Plaut, Graham and Leiner 1972; Chapman, Masterpasqua and Lore 1976; Masterpasqua, Chapman and Lore 1976; Sobrian 1976; Joffe 1977; Chapman and Stern 1978; Miley, Frank and Hoxter 1981; Miley, Blustein and Kennedy 1982; Miley 1983; Harvey and Chevins 1984; 1985; Kinsley and Bridges 1986; 1987;

[28] Fostering is when a litter is raised by a mother other than its biological mother from either the same strain or treatment as its biological mother. Crossfostering is when a litter is reared by a mother other than its biological mother and of a different strain, or from a different treatment group to that of its biological mother (Joffe 1969b).

[29] That is, a type of stressor that conforms to the criterion outlined in section 3:1 of this chapter

1988; Kinsley and Svare 1987; Peters 1988b; Kinsley et al 1989; Pohorecky et al 1989; Insel et al 1990). Consequently these will not be included in any further discussion of perinatal maternal influences.

The remaining studies, in which either fostering or crossfostering techniques were employed, divide roughly between two camps. Half reporting no postnatal maternal influences separate from prenatal influences (Thompson and Sontag 1956; Thompson 1957; Keeley 1962; Ader and Belfer 1962a; 1962b; Thompson and Quinby 1964; Joffe 1965a; 1965c; Werboff, Anderson and Haggett 1968; Archer and Blackman 1970; Herrenkohl and Whitney 1976; Politch, Herrenkohl and Gala 1978; Herrenkohl and Politch 1978; Rohner and Werboff 1979) and half reporting significant differences between prenatal and postnatal maternal influences (Hockman 1961; Denenberg, Ottinger and Stephens 1962; Thompson, Watson and Charlesworth 1962; Denenberg and Whimbey 1963; Ader and Conklin 1963; Gauron 1966; Becker and Kowall 1977; Allen and Haggett 1977; Barlow, Knight and Sullivan 1978; Fride, Soreq and Weinstock 1986; Peters 1988a).

Why some studies report postnatally mediated maternal influences and others do not, is at present unclear, but these results may well reflect the wide range of experimental procedures and types of stressors employed. Hockman (1961) for example, studied the effects of prenatal conditioned avoidance in rats and found a decrease in offspring open field activity as a function of prenatal stress; this decreased activity occured only in rats which had been raised by other experimentals or by untreated foster mothers. There was no significant decrease in the activity of experimentals reared by their own mothers. Hockman concluded that "it appears that the "stress" applied during gestation is not, by itself, sufficient to noticeably affect the offspring, but must be supplemented by the crossfostering experience" (p682). Denenberg and Whimbey (1963) in their work are even more convinced of the efficacy of the postnatal mother, considering her to be a significant contributer to their offspring effects. Thompson (1957) on the other hand stated that no behavioural differences occured as a result of postnatal rearing in his original prenatal stress experiment, however, in a later and more detailed account of the same experiment

(Thompson et al 1962) he reported that crossfostering and fostering did interact significantly with the prenatal treatments to produce the following effects on open field behaviour. At the age of 30-40 days, crossfostering increased the activity of experimentals and lowered that of controls, whereas fostering increased the activity of both experimentals and controls. In the later series of tests, carried out at 130-140 days, crossfostering decreased the activity of the experimentals and decreased that of controls, fostering doing the opposite.

According to Archer and Blackman (1971), "the reason that Thompson did not attach much importance to controlling for postnatal influences was that, in contrast to the results of Hockman and Ader and Conklin, he found that differences in postnatal rearing did not effect the general direction of the response" (p201). This Ader and Conklin (1963) paper that Archer and Blackman refer to is one in which they investigated the effects of handling during pregnancy on offspring behaviour, using both fostering and crossfostering to separate out postnatal effects. Unlike Hockman, these authors found no significant differences as a result of postnatal treatments in the open field, in an emergence test, effects of the prenatal treatment varied both as a function of age and postnatal treatment. Female offspring from the experimental group showed significantly shorter latencies than control offspring only if they were raised with their own mothers, the opposite occuring with males. In a third stress procedure, shock traumatisation, Gauron (1966) also found a significant interaction between sex of offspring and postnatal mother, this time in an open field. In his discussion, he points out that his work is consistent with Denenberg and Whimbey's (1963) and that "implications regarding offspring behavior must take into account constitutional (including physiological and biochemical elements) and environmental factors and the interaction of the two" (p223).

As can be seen from the above examples, this literature is complex and to date no consistent trend is apparent from the results. It seems that fostering and crossfostering can influence later behaviour, but the precise determinants of the particular responses remain unclear. What is interesting from the point of view of the present thesis, however, is that perinatal maternal

influences have been reported in the literature, and that maternal influences manifest themselves both in untreated mothers as evidenced by the earlier reviews and in experimentally manipulated dams.

To conclude, this present section addressed two questions; does the perinatal behaviour of the mother affect her offspring and does this continue in mothers that have themselves been stimulated? The answer to both of these questions is yes, although mediating mechanisms are as yet unclear.

3:5 SUMMARY OF CHAPTER

The purpose of the present chapter was to review all those studies in which the effects of manipulation of the mother were manifested in her offspring. The nature of the maternal treatment was narrowed down to include only those stressors which *indirectly* affected the offspring, through their effects on the mother herself, thus paralleling the procedure employed in the present thesis. Time of occurence of the stressor was taken into account, with influences occuring prior to pregnancy, prenatally and perinatally being described in some detail. The literature covered in this review leads to an overall conclusion that manipulating the mother has profound effects on the physiology, behaviour and development of her offspring, but that the nature of the stressor, the developmental period in which it occurs, the nature of the dependant variables measured, as well as age, sex and strain of animal all contribute differentially to the observed effects.

CHAPTER FOUR: GENERAL METHODOLOGY

4:1 SUBJECTS: ORIGIN, BREEDING AND MAINTENANCE

The subjects used in this thesis, bred at Goldsmiths' College, University of London, from stock obtained from Harlan Olac Ltd. (formerly Olac 1976 Ltd.) were outbred Hsd/Ola:Lister Hooded rats SPF [1] Category 3. The choice of laboratory rats as subjects reflects their use in all of the studies exploring the effects of differential maternal environments on offspring that have appeared in the literature to date (see chapter one). The decision to use "home-bred" animals was based on two factors.

Firstly, as Denenberg (1977) has pointed out, the production and management of subjects for experimental purposes is a "serious matter" and generating animals for early experience research must be carefully controlled. Specifically, the need for homogenous females has been experimentally documented by Denenberg and Whimbey (1963) and Denenberg and Rosenberg (1967), who have shown that mothers and grandmothers who have had differential handling experiences in early life will produce heterogenous offspring and grandoffspring. Furthermore, Denenberg (1977) has cautioned against purchasing pregnant females. As he says "it is immediately obvious that there must be enormous stresses placed upon an animal when it is removed from its typical environment, placed into a carton with other animals, sent by truck, rail, bus, or plane to another destination and, eventually, introduced into a strange laboratory where lighting cycles, food, ambient temperature, cleaning schedules and cages are probably very different from what these animals were used to in the commercial colony from which they were purchased" (p132). As reviewed in chapter three, prenatal stress affects the offspring (Joffe 1969a; 1969b) and raises serious questions about the value and validity of any experiment using animals obtained in this manner. Indeed, Denenberg recommends that researchers should "purchase adult females who are homogenous with respect to parity and age, and who have not been used in any experiment. After they arrive they should be allowed 2 to 4 weeks to settle into the new laboratory, after which they can be bred and their offspring used for early experience studies" (p132). This was

[1] SPF=Specified Pathogen Free, that is free of most pathogens.

taken into account in the present thesis, where all experimental animals were bred from an earlier generation bought in according to this recommendation. Indeed Denenberg's caution has recently been justified by Sachs and Lumia (1981) who have examined the effects of stress due to the shipment of pregnant animals and declared it to be a confounding variable in developmental research, leading them to "join those who have foresworn the technique" (p169).

The second and arguably more important reason for breeding the animals used in this thesis in Goldsmiths' laboratory was more pragmatic, reflecting the nature of the present research in which a complicated breeding programme extending over four generations was undertaken. Coupled with this programme, were periods of manipulation of the animals which could not have been undertaken at a breeding farm. This procedure will be described in some detail below, once the origin and breeding of the animals at Harlan Olac Ltd is outlined.

4:1:1 Origin:

The origin of the outbred Hsd/Ola:Hooded Lister rat dates back to 1932, when the Medical Research Council (MRC:Mill Hill) obtained stock from ICI, maintaining a closed colony from which Olac purchased their first animals in 1969. These animals were caesarian derived from a randomised stock, with further purchases occuring in 1972, 1979 and 1981. Described as a Black Hooded rat with fairly good breeding performance and slow growth, these animals are both easy to handle and commonly used in the literature.

4:1:2 Breeding: Harlan Olac Ltd:

Harlan Olac employ a stringent random mating system to perpetuate the outbred stock, which consists of the following procedure. Five females housed in a harem are covered by one male over a three-day period. Once pregnancy is confirmed, females are housed separately in parturition cages, until the birth of their litters. The average littersize is nine, but within the first week post

partum, litters are fostered by sex into groups of eight. Animals are cleaned out and resexed once a week until weaning which occurs at approximately 21 days. During this time, animals are maintained on an ad libitum diet (Labsure CRN nuts), in a colony room kept at 21° C, with a constant humidity of 50-60%. A 12 hour light-dark cycle is also maintained.

Harlan Olac Ltd. also maintain strict control over weights of animals. Their weight charts were employed in the present thesis to ensure that the deprivation schedules used in the experimental studies did not interfere unduly with the normal growth patterns of Hooded Lister rats. The relevant growth/weight information is detailed in Table 4:1.

AGE IN DAYS	AGE IN WEEKS	MALE AVERAGE WEIGHT	MALE RANGE	FEMALE AVERAGE WEIGHT	FEMALE RANGE
21	3	35.5	24-43	33.4	30-40
28	4	65.1	51-80	59.3	49-69
35	5	109.0	91-133	92.6	60-105
42	6	159.8	138-188	122.9	101-140
49	7	204.1	179-236	143.6	115-161
56	8	241.6	214-279	157.0	128-179
63	9	271.2	243-307	170.1	139-193
70	10	295.1	269-339	182.2	155-207
77	11	318.1	283-363	191.2	163-220
84	12	334.1	294-382	199.5	170-225

Table 4:1 Weight chart (in grammes) for Hooded Lister rat obtained from Harlan Olac Ltd.

4:1:3 Breeding: Goldsmiths' College:

As the present thesis required that a breeding programme extending over four generations be undertaken, the following terminologies were employed to describe the generations of animals involved and to delineate their backgrounds. (These designations will be continued throughout the experimental chapters.)

- F0: Male and female rats obtained from Harlan Olac Ltd: Breeding stock
- F1: Offspring of F0 animals, laboratory bred and raised: Maternal Generation
- F2: Offspring of F1 animals, laboratory bred and raised: Offspring Generation
- F3: Offspring of F2 animals, laboratory bred and raised: Grandoffspring Generation

Of particular importance to the present research is the F1 generation, as this was the group that would provide the future mothers and grandmothers of the experimental animals, F2 and F3 generations respectively. This F1 group was also the one to receive the maternal manipulation.

The breeding procedure employed in this thesis was as follows: the F0 females arrived from Harlan Olac Ltd, aged 10-12 weeks (175-200g) and were housed in groups of five in standard paturition cages, available from North Kent Plastic Cages Ltd, for two weeks prior to being mated. This was to allow them to settle into the laboratory routine, as recommended by Denenberg (1977). Two females were then placed into a group holding cage and a male rat introduced [2]. These animals were left undisturbed for five days, the females then being individually housed in parturition cages and placed in a quiet part of the colony room behind partitions, where they remained throughout the remainder of their pregnancies. During this period, food and water were replenished twice weekly and the animals cleaned out twice (Days 12 and 19 of gestation). Care was taken to reduce all noise in the colony room at this time and the animals were disturbed as little as possible. During the parturition period, cages were checked daily for litters, and dates of birth recorded. Once the litters had arrived, the animals were left undisturbed (apart from topping up food hoppers and refilling water bottles) until the pups were 19-21 days old. At this point the F0 dams were removed and the litters weighed and sexed. Female pups (F1) were allocated to one of the three experimental environments employed in this thesis, namely the Superenriched (SEC), Standard (SC) and Impoverished (IC) conditions (see section 4:2) using a split litter design, so that each litter was equally represented in each environment. Split-litter techniques as a method of control have been discussed in some detail by Henderson (1968) and are recommended as a way of eliminating systematic bias from individual experiments.

The F1 generation remained in their experimental conditions for nine weeks until they were sexually mature (82-84 days old). At this point, the animals were removed from the environments,

[2] The decision to mate two females with one male, rather than use the harem method of five females to one male was taken for two reasons. Firstly, this method ensured that if any infertile males or animals with poor copulatory performance were used as studs, few females would be affected. Additionally, the use of more males would increase the gene pool, maintaining an outbred colony.

placed in parturition cages in pairs and a male rat introduced. These males were bought in especially for breeding purposes and were only ever used once so as to maintain an outbred colony. Furthermore, these animals were always group housed, as keeping males in isolation has been found to reduce copulatory behaviour (Dunlap, Zadina and Gougis 1978). These animals were then left undisturbed for five days and the general procedure observed for the F0 generation carried out.

Offspring (F2) of this breeding were weaned at approximately 19-21 days, the litters weighed, sexed and, depending on the experimental requirements, individual F2 animals were weighed and individually housed in standard solid-bottom experimental cages (also available from North Kent Pastic Cages Ltd). For study two (chapter six), a further generation of animals was required, consequently F2 females were weaned and placed in the Standard Condition (SC) cages (see below for details) where they were maintained for nine weeks until sexually mature, at which point the established mating, gestation and parturition procedures described above were implemented. Their offspring (F3) were weaned at 19-21 days old and as before, litters were weighed and sexed, then the individual animals were weighed and housed in the single experimental cages employed throughout this research.

4:1:4 General Animal Housing and Maintenance:

All the animals used in this research, both for breeding and experimental purposes were kept in the colony room at Goldsmiths' College, where a constant temperature of 18-22^{o} C and humidity of 50% are maintained, as is a 12 hour light-dark cycle (8am-8pm). All treatments and maintenance were carried out, as far as possible, by the same experimenter. All the animals in this research were maintained on Oxoid Breeding Diet [3], and unless otherwise stated, both food and water were available ad libitum. Animals were cleaned out twice a week, care being taken to minimise handling animals during this procedure.

[3] This contains oil 4%, protein 21%, fibre 4%, ash 5%, calcium 0.8%, phosphorus 0.63%, sodium 0.31%, salt 0.83%, potassium 0.85%, starches 33.5%, sugars 5% per kilo.

4:2 DIFFERENTIAL ENVIRONMENTS

Before describing the differential environments employed in this thesis, the nature of the enriched environment employed in the present work must be considered. Typically, in the literature, the "enriched" "complex" or "free" environment consists of ten or more animals living in a large cage, in which there is a variety of stimulus objects (Rosenzweig and Bennett 1977). However, there have been many variations on this theme. For instance, in their initial experiments, the Berkeley group employed an "environmental complexity and training" cage (Krech et al 1960), which contained a small wooden maze and two wooden objects selected each day from a selection of seven items. In subsequent work, more objects were encorporated into the environment (Rosenzweig and Bennett 1969). In addition, the rats were also placed in the field of a Hebb-Williams apparatus 75cm x 75cm, where the pattern of barriers was changed daily. In 1965, the Berkeley animals also received one or two trials a day in various standardised mazes, for sugar pellet rewards, but it was found that this procedure could be omitted without affecting the enrichment effects and the designation "environmental complexity and training" was changed to "enriched condition".

"Superenriched" environments have also been employed. For example the enriched condition employed by Ferchmin and his colleagues (Ferchmin and Eterovic 1986) involved rotating animals twice a day amongst four cages, some of which were larger than the standard Berkeley EC and offered more opportunity for climbing. Probably the best known superenriched environment, however, is the one designed by Clive Kuenzle and Alois Knusel (1974). This environment was developed to mitigate the recurrent criticism that the traditional EC mostly improves an animal's motor performance, but does not provide genuine learning situations. Kuenzle and Knusel housed a group of 70 animals in two large cages, provided with various stimulus objects and connected by tunnels. Each day, for 29 days, food was placed in one of the cages and water in the other, so that the rats had to travel between the two cages. Gradually the rats were made to solve problems

and to climb ropes, or jump from one platform to another to traverse the tunnels [4]. Davenport (1976) has also used a superenriched environment, using a modified version of Kuenzle and Knusel's SEC, which consisted of three standard wire mesh cages, with interconnecting tunnels and containing a variety of objects and a maze aligned with a food (or water) trough which was mounted on the outside of the centre compartment's front door. Animals were housed in groups of 45 and were required to learn various ways to obtain food and water, as the apparatus features were changed every third day.

Finally, another and quite different method by which the effects of differential environments have been enhanced, is through the use of a "semi-natural" environment (SNE) described by Bennett (1976) and Rosenzweig et al (1978). In the SNE, rats lived out of doors, in a 9 sq.m enclosure with a dirt floor 60 cm deep. Rats put into the SNE at 40 days of age were found to have moderate, but significant increases in cerebral weight measures as compared with animals maintained in the standard EC.

Given that in the present design animals were to be maintained in their differential environments from weaning until they were sexually mature, that is for approximately 65 days and may well habituate to the enriched environment (Rosenzweig, Bennett and Diamond 1972) and that superenrichment appears to enhance enrichment effects, a modified version of a superenriched environment was employed in the present thesis. In addition, differences in brain weight (total cortex and subcortex) between EC/IC animals have tended to be less significant after 80 days of environmental experience, than after 30 days (Rosenzweig and Bennett 1968; 1972), a time period similar to that employed in this work. Since it has been suggested that this may also be due to the fact that the animals were beginning to habituate to the enriched environment (Rosenzweig, Bennett and Diamond 1972), in the present experiments a more complex environment was employed, to alleviate this problem.

The impoverished condition used in the present work, was based on the IC used by the Berkeley

[4] N.B. In three succesive experiments, the SEC rats were found to have significantly greater cerebral length, weight of occipital cortex and ChE/AChE ratio, than animals raised in the original EC.

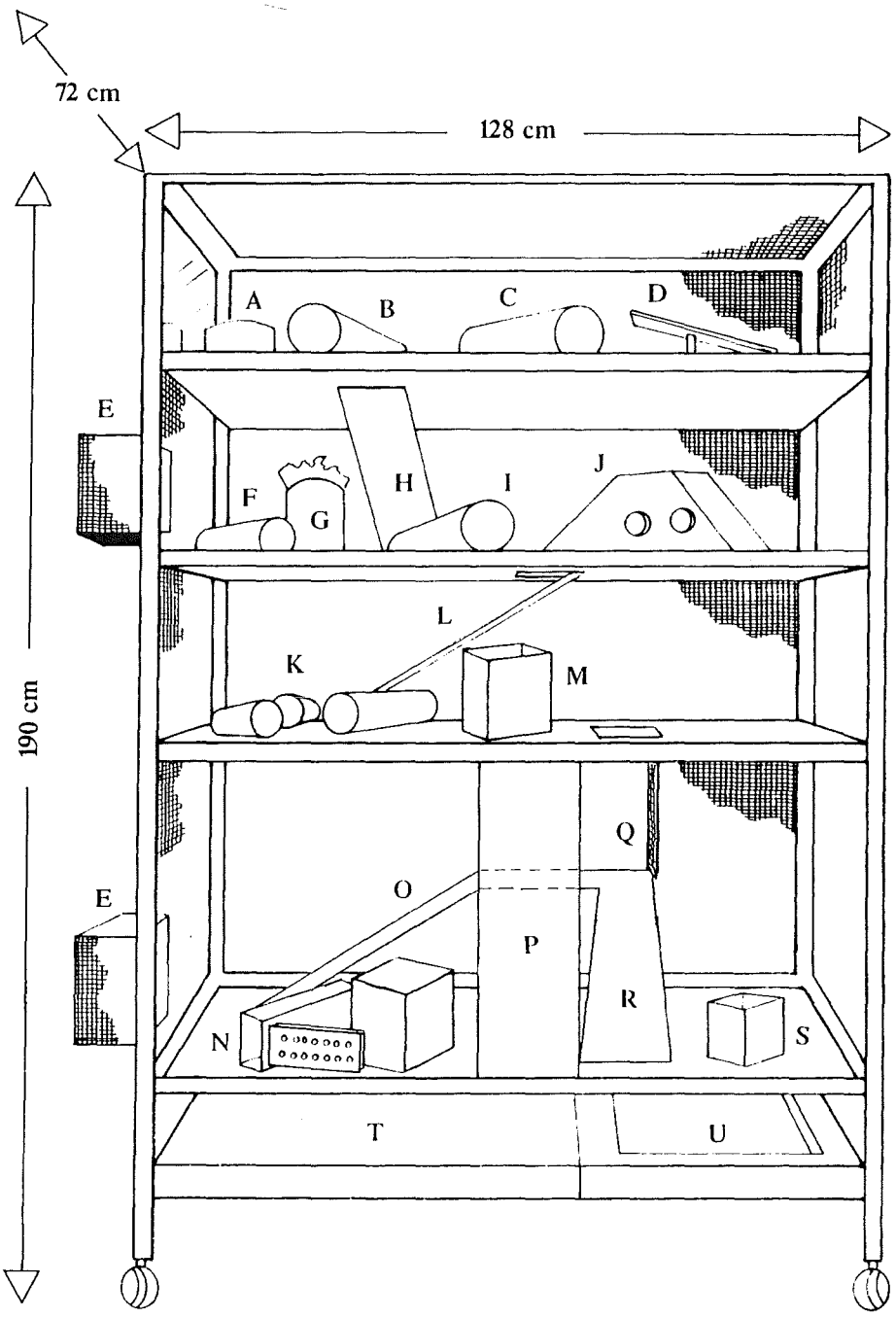
group (Diamond Johnson and Ingham 1971). In addition, a standard housing condition (SC) was also employed, to provide a control referent, as advocated amongst others by Kiyono et al (1985). Rather than maintaining animals in groups of three, however, as the Japanese researchers did, the present SC comprised animals housed in groups of five. This was because Krech et al (1966) have pointed out that "the socially housed animals are somewhat impoverished, since they spend all their time in small cages with only two cagemates". Consequently, in the present work, larger groups of animals were housed together and in larger cages than those of the impoverished condition.

The particular environments employed in this thesis, namely the Superenriched Condition (SEC), the Standard Condition (SC) and the Impoverished Condition (IC) are described in some detail below.

4:2:1 The Superenriched Environment:

As can be seen from Diagram 4:1, the Superenriched environment consisted of a large custom built cage (190x128x72cm) horizontally subdivided into four sections, the bottom section being vertically subdivided into two compartments. One of these compartments was equivalent in size to the traditional enriched environment used by Rosenzweig et al (Bennett, Diamond, Krech and Rosenzweig 1964). Access to the sections was by means of a series of interconnecting shelves and runways. Construction materials included a basic skeleton built of black "speed-frame", covered with 1cm wire mesh. To allow access to all the horizontal compartments, the front of the cage was made of clear perspex doors, opening centrally. Inside, the upper levels were connected by aluminium shelves, covered with wire mesh to prevent the animals from slipping. One side of the top level was made of mirrored perspex, to add variety.

The environment contained a variety of toys, some of which were permanent fixtures, including a "teeter-totter" in grey and clear perspex, attatched to the top level; a three dimensional slide in clear and textured perspex incorporating a mesh wall and typically found on the third level;



KEY	
A	Clear perspex tube by mirror
B	Aluminium tube
C	Coloured perspex tube
D	Teeter-totter
E	Wire mesh food hopper
F	Clear perspex tube
G	Perspex box with tissue paper
H	Aluminium connecting ramp
I	Coloured perspex tube
J	Clear perspex box with holes for access
K	Various tubes

KEY	
L	Aluminium connecting ramp
M	Coloured perspex box
N	Various perspex boxes
O	Connecting ramp
P	Board for cage information
Q	Mesh connecting sections
R	Connecting ramp
S	Mirrored perspex box
T	Removable litter tray
U	Removable "swimming pool"

Diagram 4:1 The Superenriched environment

and a water trough, again in perspex, built into the bottom level. This "swimming pool" was either filled with water or sawdust. In addition a variety of other toys were rotated around the cage, new and interesting combinations of tubes, cubes, wood, ping pong balls and paper being introduced when the inside of the cage was cleaned out twice a week. Externally mounted food hoppers, water bottles and litter trays were also incorporated into the design of this environment.

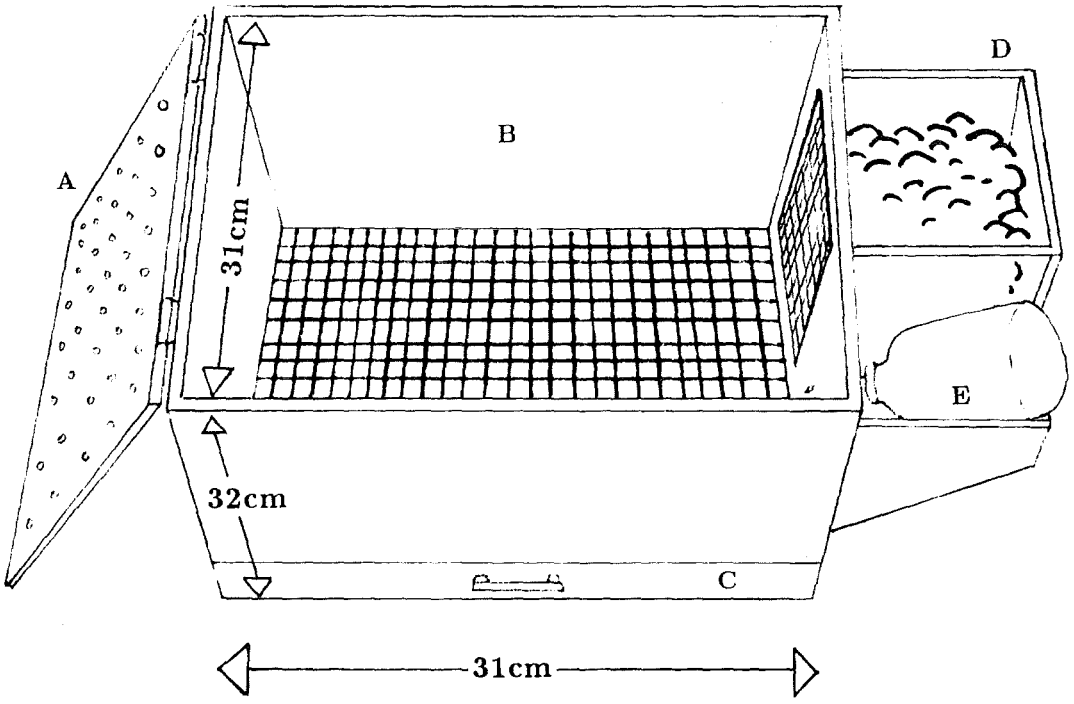
At any given time, following Davenport's (1976) example, at least 45 females were housed in this environment, being confined to the bottom compartment for the first week following weaning, until they were big enough to climb about the whole cage. To maximise external stimulation, the SEC was placed in the busiest part of the colony room.

4:2:2 The Standard Environment:

Available from North Kent Plastic Cages Ltd., these consisted of standard "group housing" laboratory cages. Made of white polypropylene (56x38x18cm) with stainless steel lids, grids and food hoppers, these cages also had externally mounted water bottles. Animals were housed in groups of five and were cleaned out twice a week. Unlike the previous environments, the SC animals were kept in a quiet part of the colony room, near the breeding colony. Food and water were available ad libitum.

4:2:3 The Impoverished Environment:

Based on the impoverished environments employed by Rosenzweig and Bennett, each environment (see Diagram 4:2) consisted of a grey perspex cage (31x31x32cm) with a wire mesh floor 6cm above the base of the cage, underneath which was a fitted litter tray. The coloured perspex prevented the animals from receiving any visual stimulation, although the cage lid was of clear perspex to allow in the light and 1cm diameter ventilation holes. As with the other environments, food hoppers and water bottles were externally mounted, and the cages were cleaned out twice a



KEY	
A	Clear perspex perforated lid
B	Impoverished cage with grid bottom
C	Removable litter tray
D	Food hopper
E	Water bottle

Diagram 4:2
Schematic representation of
the Impoverished Environment

week. Animals were individually housed in these environments and to ensure minimal stimulation, IC cages were maintained on a high shelf 210cm off the ground, in a quiet part of the colony room.

4:2:4 Experimental Housing:

During the behavioural experiments to be described in the following chapters (6-10), all animals were housed in the same manner to standardise their environmental experience, namely in individual cages made of white polypropylene (38x25x18cm), with solid bottoms and external food hoppers and water bottles. These cages were bought from North Kent Plastic cages Ltd. and were mounted on racks so they could be wheeled to the experimental rooms when required.

In all experiments the assistance of a technician was enlisted to label the animal cages with coded numbers, so that the experimenter did not know from which group the animals originated. This was to minimise experimenter bias, especially important in those behavioural tests where the experimenter was recording behaviours manually. Only after all the procedures were completed were the animals' backgrounds revealed and the data analysed.

4:3 BEHAVIOURAL TEST APPARATUS

The effects of environmental manipulations were investigated using a fairly standardised battery of tests. Justification for choice of apparatus is presented in the experimental chapters. The various types of apparatus employed are described below.

4:3:1 The Open Field:

The open field consisted of a 120cm diameter white perspex circular arena, surrounded by a 34cm high grey PVC wall, which prevented the animals from escaping. The floor was subdivided into three concentric circles of 12, 36 and 90cm radius, the circles being further subdivided into

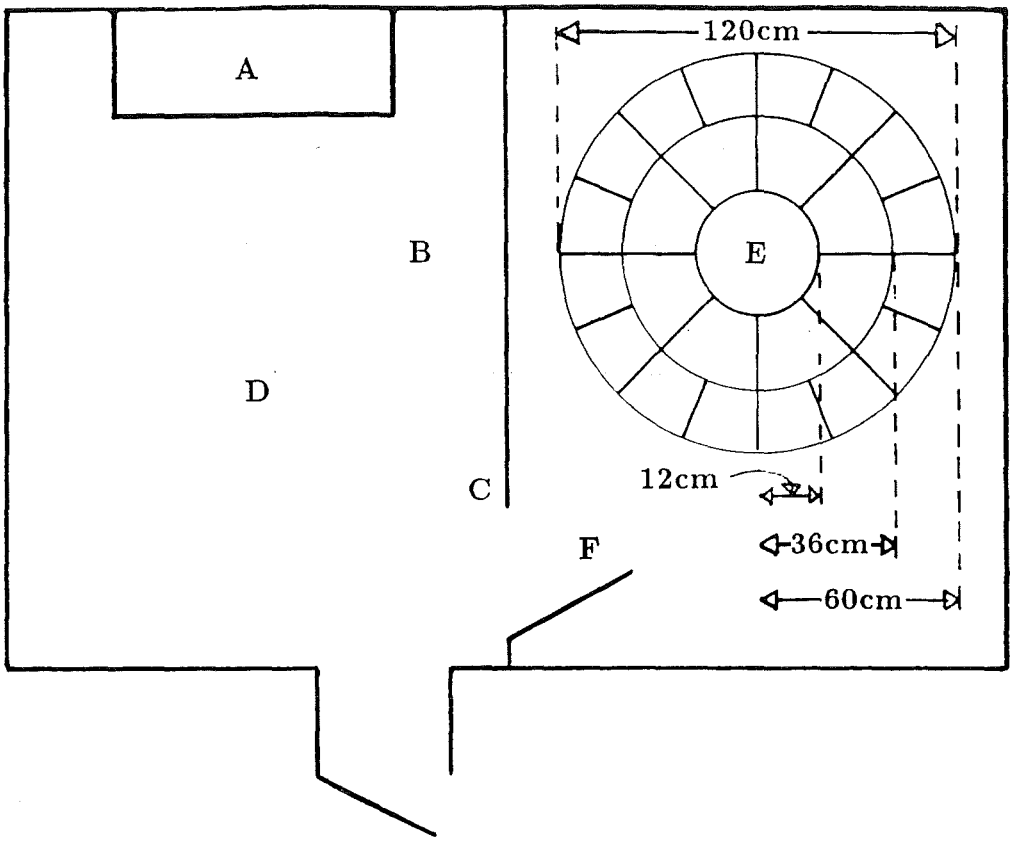
1, 8 and 16 sections respectively. The open field was situated in the centre of an experimental room, adjacent to which was an observation room in which the experimenter worked. The connecting wall included a one-way glass panel, so the animals could be observed undetected. Suspended 200cm above the field was a 60 watt light bulb, positioned in such a way as to mimimise the formation of shadows. Built into the roof of the experimental room was a video camera, connected to recording equiment and monitoring screens in the observation room, which allowed the experimenter to record all activity in the field. Measurements were taken using stopwatches and tally counters. Tego disinfectant was used to clean the apparatus between animals. This apparatus, and its position in the experimental room is detailed in Diagram 4:3.

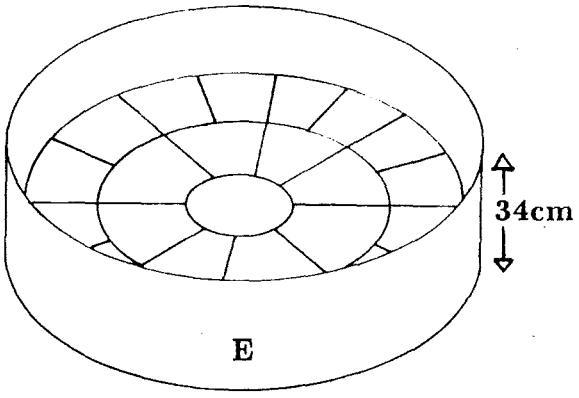
4:3:2 The Hebb-Williams Maze:

Housed in the same experimental rooms as the open field described above, the Hebb-Williams maze employed in this thesis was a modification of the apparatus designed by Rabinovitch and Rosvold (1951). In particular, as can be seen from Diagram 4:4, the maze consisted of a 76.2x76.2cm field, marked off in 12.7cm squares with moveable barriers and start and goal boxes at diagonal corners. Rather than being the traditional "closed" field, however, this version was open, with walls 34cm high, allowing the animals to rear but not to escape. The floor of the field was painted white, the walls, barriers and floor markings black and the whole apparatus was illuminated by a 60 watt light source suspended 200cm centrally above it. As with the open field, this apparatus was directly underneath a video system incorporated into the roof of the experimental chamber to allow all testing phases to be recorded. The maze was kept clean between animals, by wiping it down with Tego disinfectant.

4:3:3 Skinner Box Apparatus:

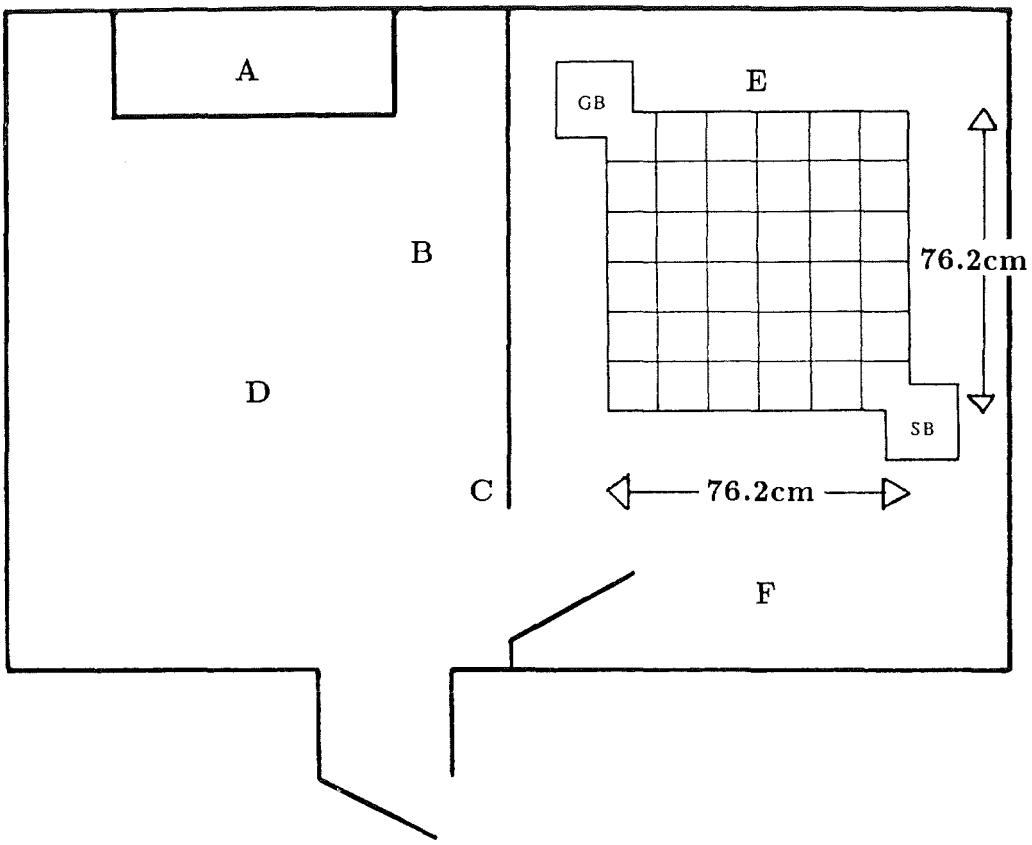
In this thesis two separate Skinner box systems were employed. These will be designated Type I and Type II, for the remainder of this report.

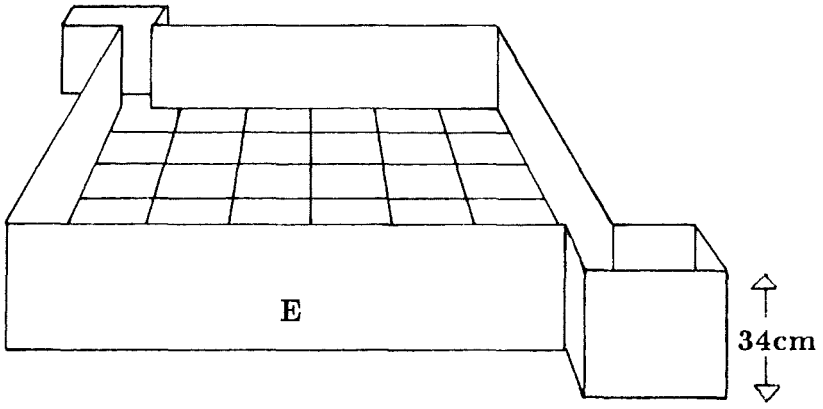




KEY	
A	Data collection table
B	Position of experimenter
C	One-way mirror
D	Observation room
E	Open Field
F	Experimental room

Diagram 4:3 Plan and schematic representation of the position of the Open Field in the experimental rooms





KEY	
A	Data collection table
B	Position of experimenter
C	One-way mirror
D	Observation room
E	Hebb-Williams maze
F	Experimental room

Diagram 4:4 Plan and schematic representation of the position of the Hebb-Williams maze in the experimental rooms

SB = Start Box; GB = Goal Box

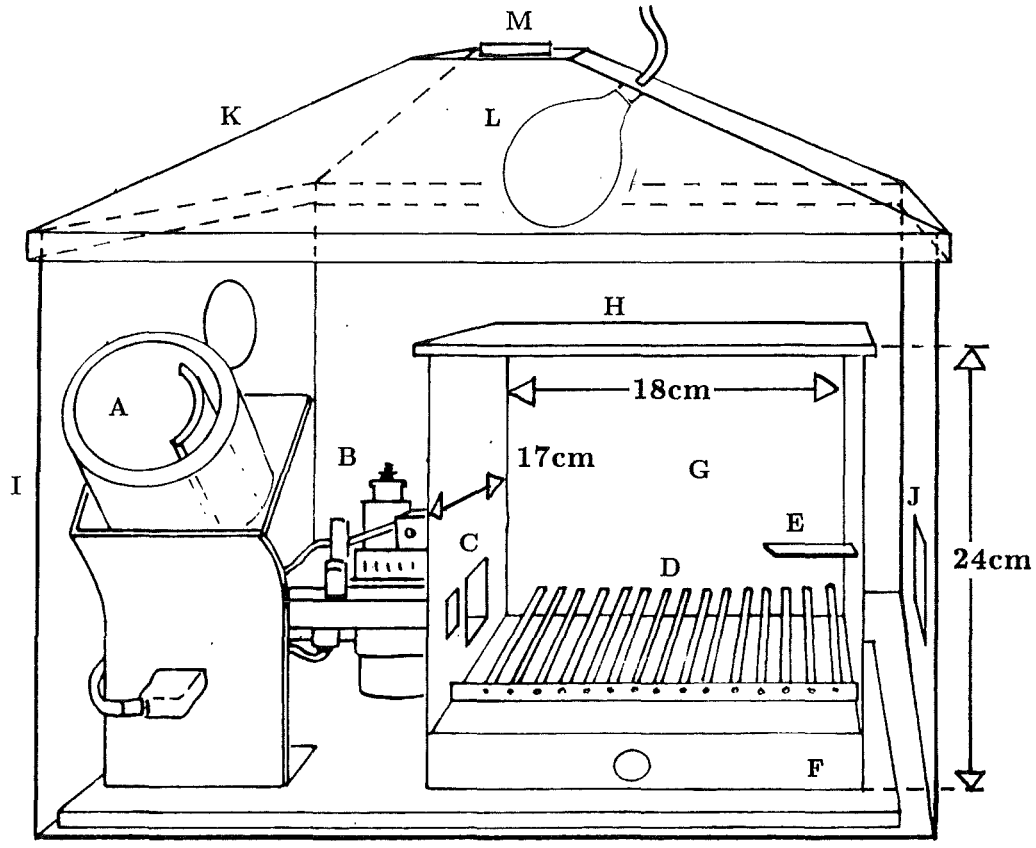
Skinner Boxes: Type I

Based on the research by Rose, Love and Dell (1986) and Rose, Dell and Love (1987) in which the nature of the reinforcement was manipulated, this apparatus comprised modified Skinner boxes, originally supplied by Camden Instruments Ltd. Made from aluminium, with a white plexiglass roof, these boxes measures 24x18x17cm and were each enclosed within sound attenuating chambers which contained both ventilation fans and lamps for providing reinforcement illumination. Food reinforcement in the form of 45mg Noyes pellets (Camden Instruments Ltd.) was also available, dispersed from a pellet dispenser built into the equipment. Because of the size of the animals, the Skinner box levers were modified so that pups could manipulate them easily. Specifically, the levers were cantilevered such that the lightest pressure depressed them and were made of light-weight aluminium.

Housed within an experimental room, the operation of the boxes was controlled by predetermined counters, with relay counters recording the number of responses and reinforcements. Session length was determined by a process timer. In addition, the lamps were connected to variable voltage metres, so as to provide, as required, different levels of illumination of the white perspex sheet covering the roof of the Skinner box. This apparatus is shown diagramatically in Diagram 4:5. Overall lighting of the experimental room was kept to a minimum, with a 60 watt red light bulb placed near the recording equipment.

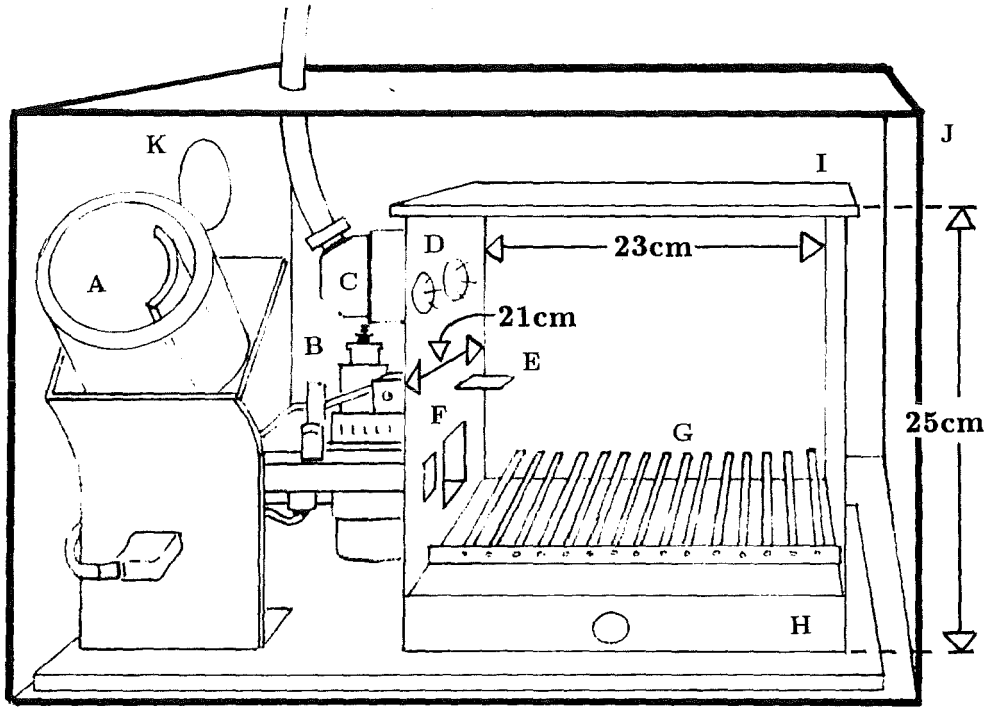
Skinner Boxes: Type II

Set up in a separate experimental room (see Diagram 4:6) from the previous equipment, this system comprised a bank of 16 standard Skinner boxes, supplied by Camden Instruments Ltd. As with the previous boxes, the levers were modified so that young animals could manipulate them easily. These boxes were also made from aluminium, but were slightly larger than those used in the Type I system, measuring 25x21x23cm. Furthermore, they had clear perspex *front*



KEY	
A	Pellet dispenser
B	Skinner box motor
C	Food tray
D	Grid bottom of cage
E	Adapted lever
F	Litter tray
G	Skinner box test chamber
H	White perspex lid
I	Sound attenuating chamber
J	Ventilation panel
K	Aluminium hood
L	Light bulb
M	Fan

Diagram 4:5 Schematic representation of the Type I Skinner Box Apparatus



KEY	
A	Pellet dispenser
B	Skinner box motor
C	Computer terminal lead
D	House light
E	Lever
F	Food tray
G	Grid bottom of cage
H	Removable litter tray
I	Skinner box test chamber
J	Sound attenuating chamber
K	Ventilation panel

Diagram 4:6
Schematic representation of
the Type II Skinner Box Apparatus

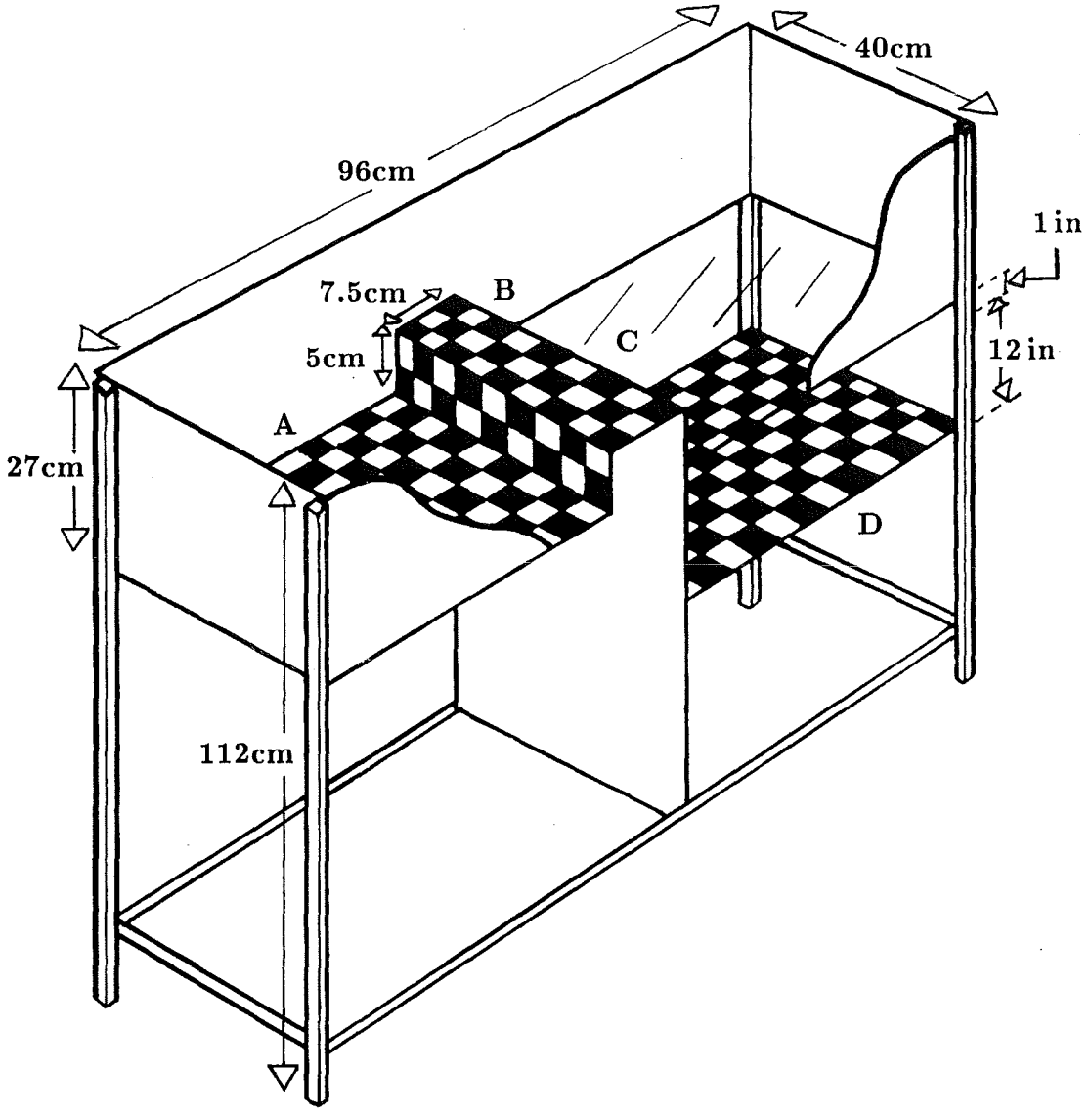
panels, rather than opening from the top. Incorporated into the Skinner box was a pellet dispenser and four house lights, all of which were empoyed as the reinforcer. Each box was enclosed in a sound insulated fan ventilated cabinet and was interfaced with an eight bit interrupt driven microcomputer (Control Universal Ltd.), whose programme (Written in interger ONLIBASIC by M.B.Curry 1982) controlled both the output to and input from the boxes, as well as recording animals' responses.

All Skinner box equipment (Type I and Type II) was checked daily and cleaned between trials, undergoing a thorough cleaning once a week.

4:3:4 The Visual Cliff Apparatus:

Based upon the apparatus described by Gibson and Walk (1960) and modified by Lamden (1985), this apparatus consisted of a black "speed-frame" structure 96cm long, 40cm wide and 27cm deep, mounted on legs such that the top of the visual cliff was 112cm off the ground. As can be seen from Diagram 4:7, from the top, the apparatus comprised a 6mm thick sheet of glass, below which was a chequerboard pattern of 2.5cm black and white squares, the pattern being immediately below the glass on the "shallow" side and on a moveable shelf on the deep side of the apparatus. The depth of this shelf could be varied beneath the clear glass, producing the "cliff" effect. Between the cliff and shallow sides, resting on the glass was a central raised platform 7.5cm wide and 5cm high, also covered in the check pattern. This barrier constituted the modification of the original cliff design, its purpose being to prevent the animals' whiskers touching the glass and influencing their visual cliff behaviour.

The apparatus was housed in an experimental room illuminated by two anglepoise lamps fitted with 40 watt bulbs, carefully positioned so as to avoid any reflection on the glass surface. As with the Hebb-Williams and open field apparatus, the visual cliff was cleaned between trials with Tego disinfectant.



KEY	
A	Checker-board shallow side
B	Platform
C	Glass covering deep side
D	Moveable checker-board shelf

Diagram 4:7 Schematic representation of the Visual Cliff Apparatus

4:4 DRUG STUDY

Chapter eight of this thesis involved manipulation of arousal levels through the use of amphetamine. As this procedure was only employed in this one study, details of drug preparation, injection and handling of animals will be discussed in detail in the methodology section of that study, rather than here.

4:5 DATA ANALYSIS

Before describing the statistical analyses employed in this thesis, two design features which were incorporated into the breeding procedure, and which by their very nature may influence the choice of statistical test, require further elucidation.

As mentioned in section 4:1:3, none of the litters bred for this present research were disturbed between parturition and weaning. That is, no fostering or cross-fostering [5], or culling of litters was employed, despite the fact that these procedures have been recommended by Joffe (1969a; 1969b) as postnatal controls in early experiential research.

The decision not to foster was based on several considerations. Firstly, the procedure requires that litters be handled, which in itself has been found to have profound effects on both the animals' physiology and behaviour, and on the behaviour of their offspring and grandoffspring (see chapter three). Secondly, it was felt that leaving litters with their natural mothers would maximise any maternal influences, a methodology that has been employed elsewhere in the literature (Moyer, Herrenkohl and Jacobowitz 1978). Finally, as Moore and Power (1986) have pointed out, because prenatally stressed pups elicit differential maternal care, cross-fostering does not eliminate the possibility that maternal stimulation may mediate some prenatal stress effects. This questions the validity of crossfostering as a useful technique in this type of research, anyway.

[5] This technique involves removing either a proportion of the litter or whole litters from their natural mothers and fostering them to another post-partum dam from the same experimental condition or crossfostering them to a dam from a control group.

With respect to the decision not to cull litters to obviate littersize effects, again several factors were taken into account. As with fostering, culling requires that the litters be handled, a procedure which may well interact with the maternal manipulation employed in the present thesis (Denenberg and Rosenberg 1967). Secondly, although the littersize effect on an animal's behaviour has been well documented in the literature (Seitz 1954; 1958; Milkovic, Paunovic and Joffe 1976; Cross and LaBarba 1978; Chapman and Stern 1979), not all manipulations of littersize have had significant effects (Broadhurst and Levine 1963; Fuemm and Driscoll 1981), leading Denenberg (1963) to suggest that it might be a strain specific phenomenon. To date, no data on the effects of manipulation of the littersize of Hooded Lister rats on the behaviour of these animals exists in the literature. However, a third and more influential factor does support the decision not to cull, namely that within the literature there are a variety of *statistical* procedures available to control for littersize effects, which remove the necessity of culling.

The most commonly cited method is one developed by Abbey and Howard (1973) in which the means of the litters rather than the measurements of individuals are entered into the analysis. This has the effect of removing any correlation of observations within litters. Furthermore, as Abbey and Howard point out "when differences between litters contribute a large part of the total variance, and when the experimental manipulation has to include the litter as a whole, a larger number of litters should be studied with a small number of individuals from each litter" (p332). However, there are exceptions to this principle. For example, in postnatal studies "when pairs of treated and control animals from the same litter can be used, the effects of variations between litters can be balanced in most cases" (p332). This use of the split-litter technique was employed whenever possible in the present thesis.

One obvious practical problem with Abbey and Howard's recommendation is that it requires large groups of litters and a high level of wastage of animals. An alternative method is to employ a specific *number* of animals from each litter, in each test. As Fride, Dan, Feldon, Halevy and Weinstock (1986) have commented, in their experiment "in order to prevent litter effects no more

than two male and/or female littermates were used for a particular test" (p682). This procedure was taken up in the present thesis and wherever possible, in experiments involving F2 and F3 generations where a split litter design was not possible, equal numbers of animals from each litter were assigned to each behavioural test.

Finally, as Denenberg (1977) has noted, if there are no significant litter effects, then it is legitimate to use the individual subject as the unit for statistical analysis. As he says "the statement that "there are no significant litter effects" is equivalent to stating that there is no correlation among littermates with respect to end points being evaluated" (p140). To put this into somewhat more formal statistical language, "we have what is called a *nested* or *hierarchical* (sic) design in which there are two random variables: (1) Litters within Treatments and (2) Subjects within Litters within Treatments. The former term has to be used as the measure of error variance unless a statistical test establishes that this is not a significant source of variance" (p140).

In order to establish that litters within treatments is not a significant source of variance, a technique employed in the "recovery of function" literature (Rose, Davey, Love and Dell 1987) was adopted for this present research. In this literature, size of lesion may well influence any results. In order to check that lesion size effects are not significant, that is that there are no significant differences between lesioned groups exposed to differential environments, it is common to perform an analysis of variance on the percentage of lesion per animal across all experimental groups. If no differences emerge, which is typically the case, analyses of behavioural measures are undertaken, without incorporating lesion size as a variable. With respect to the present research, the littersize of each animal from the three maternal groups provided the unit of analysis. If no significant differences emerged between the groups [6], then, as recommended by Denenberg (1977) individual subjects can be used in future analyses. If however, littersize effects are found to be significant, then further statistical checks need to be imposed.

One method which can be employed under these circumstances, is to include littersize as a

[6] That is any contribution of littersize effects to the behaviour of offspring was equivalent for the three experimental groups.

covarying factor in the analysis (Harrington 1968). The only problem with this method lies in the fact that techniques based on covariance matrices are not as robust as those based on analysis of variance (Harrington 1968). Hence homogeneity of variance is a matter of concern and so in the few situations where ANCOVA was employed in this thesis, Bartlett's test of homogeneity of variance was employed (Winer 1971) and where appropriate, data were transformed.

Statistical Analyses Employed

Specific details of methods of data analysis will be discussed in the context of individual experiments. However, the most frequently used methods were Analysis of Variance and the Chi-Square Distribution (Winer 1971; Kirk 1968).

ANALYSIS OF VARIANCE

In testing the experimental hypotheses, certain assumptions connected with the performance of analysis of variance (ANOVA) need to be considered. Firstly, as a parametric test, ANOVA presumes that the data comes from a normal distribution and more specifically that the frequency distribution of scores is not skewed. In addition, it is assumed that variance among the groups is not significantly different. Put more formally, hypothesis testing based on the F distribution as the theoretical model involves the following asumptions:

- Observations are drawn from normally distributed populations.
- Observations represent random samples from populations.
- Variances of populations are equal.
- Numerator and denominator of F ratio are independent.

(Kirk 1968).

Deviations from normality may result in the loss of power in the F test, with a concomitant reduction in efficiency of estimating main and treatment effects (Cochran 1947). Typically data which are not normally distributed can be made more nearly normal by employing either a square-root transformation or a logarithmic transformation. However, as Robbins (1977) has pointed out "the gain in power of the F test resulting from a normal transformation of raw scores is at the expense of testing a different null hypothesis from the one specified before transformation. If the prior transformation has psychological validity and fits a specified model describing the psychological data, then, of course, its use is justifiable. In many instances, though, the psychological data are transformed to meet the requirements of the analysis of variance" (p 57). Consequently Robbins (1977) recommends "that data remain untransformed for the analysis of variance" (p58) and further more that "it is considered that the loss of efficiency in the statistical test is a small price to pay for results that have greater psychological validity". Indeed, in most cases F tests are sufficiently robust to withstand large-scale deviations from normality of the data (Winer 1962) and as Kirk (1968) has said, "in general, unless the departure from normality is so extreme (it) will have little effect on the probability associated with the test of significance" (p61). Furthermore, with regard to the assumption of population-error variances, Kirk (1968) has noted that "since the F distribution is so robust with respect to violation of the assumption of homogeneity of error variance, it is not customary to test this assumption routinely" (p 62).

In the present thesis, although main effects provided an important source of information about the psychological significance of the data, for much of the work, the patterns of behaviour of the animals manifest in interactions were of particular relevance. Consequently, following Robbins' (1977) advice, in none of the ANOVA's were any data transformed.

In addition, in the experimental chapters in which analysis of variance performed on the data demonstrated significant differences between more than two groups, Newman-Keuls' Multiple-Range Tests (Bruning and Kintz 1977) were employed.

NEWMAN-KEULS MULTIPLE RANGE TEST

With more complex analyses of variance, such as were typically employed in the present thesis, it is often necessary to determine which specific means differ from each other. There are several tests available to deal with such "simple effects". There is some controversy, however, regarding which of these tests is most appropriate, but of the tests which can be used, only those which correct for the probability for choosing the comparisons to be made after the main analysis has been completed should be employed. These include Scheffe's test, the Tukey test, Duncan's Multiple Range test and the Newman-Keuls' Multiple Range test. Of these, only the latter two adjust the size of the critical difference depending on whether the two means being compared are adjacent or not. Of these, according to Bruning and Kintz (1977), "the mathematical bases for the Newman-Keuls' tables are more defensible than those for Duncan's" (p119) consequently in the present thesis all post hoc comparisons employed the Newman-Keuls test.

CHI-SQUARE DISTRIBUTION

The Chi-Square Distribution (χ^2) is used to analyse frequency data, and has the following assumptions:

- the χ^2 distribution is a continuous curve and the observed frequencies used in its estimation take on whole number vales. The smaller the sample size, the worse the fit to this continuous distribution and below a certain size, χ^2 should not be used. Typically it is recommended (Robson 1973) that this method be rejected if one or more of the expected frequencies falls below five.

- Each observation is independent of each other and every other observation.

DATA ANALYSIS AND GRAPHICS

Throughout this research, whenever possible, computerised statistical and graphical packages were used, details of individual analyses being found in the results sections of the relevant experiments and appendices. Briefly, however, the following packages were employed:

- SPSSX (3rd Edition): Statistical Package for the Social Sciences (Spss Inc. Chicago USA) 1988: Run on a mainframe computer: Digital's VAX/VMS.
- ANOVA programme "ANOVA ON APPLE" based on UCSD PASCAL II.I, written in PASCAL I.I for the Apple II microcomputer (Apple Computer Inc. 1979), by S.Fearnley (Oxford Polytechnic 17/3/82) and modified by Dr R.J.H.Russell (University of London:Goldsmiths' College) in 1982.
- Latex, a Document Preparation System (Version 2.09) released 19 April 1986, written by L. Lamport, for use with VAX/VMS.
- Apple Mac: Cricket Draw 1:1:1 and Cricket Graph 1:3:2, Computerised Graphics programmes employed in all the colour diagrams.

CHAPTER FIVE: STUDY ONE

EFFECTS OF DIRECT EXPOSURE TO SEC, SC AND IC ON BEHAVIOUR IN MALE, VIRGIN FEMALE AND POSTPARTUM FEMALE RATS.

5:1 GENERAL INTRODUCTION

Before addressing the main question of this thesis, namely what are the effects of differential maternal environments on offspring behaviour, a couple of practical issues had to be resolved. These provide the focus of this first experimental chapter.

Firstly, the type of enriched environment employed in this thesis is rare in the literature, only having been employed once before (Rose, Dell and Love 1985a). Moreover, it is best characterised as a superenriched environment (SEC), rather than the more conventional type of enriched environment employed by Rosenzweig and his colleagues. Although the effects of the more traditional form of enrichment and impoverishment have been well documented in the literature (see chapters 1 and 2), it was necessary to provide a baseline of behavioural effects in animals directly exposed to the environments (and in particular the SEC) employed in this thesis, against which to compare their offspring. These requirements were met in experiment one of this chapter.

Secondly, within the EC/IC literature, animals are typically housed in environments for 30 days following weaning. In the methodology employed in this thesis, however, animals were to be exposed to differential environments for nine weeks, then mated, experience pregnancy, parturition and rearing of offspring. These additional procedures might well have interacted with the environmental experience of these animals and thus influenced the behaviour of their offspring. Consequently, experiment two was designed to investigate the effects of both length of environmental experience and the effects of pregnancy and mothering on the behaviour of these animals, to ensure that the expected SEC, SC and IC differences were still apparent post partum.

5:2 EXPERIMENT ONE

5:2:1 INTRODUCTION

Given the fact that in the present research animals were to be housed for nine weeks in differential environments, a length of time in which they might conceivably habituate to a traditional EC and given that on balance more studies have found beneficial effects of SEC (Sturgeon and Reid 1971; Brown and King 1971; Kuenzle and Knusel 1974; Bennett 1976; Davenport 1976) than not (Davenport 1976; Rose, Dell and Love 1985a), it was decided to employ a superenriched environment in the present thesis, to maximise enrichment effects.

One obvious concern, however, relating to this decision, was the lack of beneficial effects of SEC reported by Davenport (1976) and Rose et al (1985a). The latter authors in particular presented this current work with something of a dilemma, as they had employed the same SEC as was to be used in the present thesis. They had housed eleven animals in each of five different environments including the SEC, as well as a traditional EC, an SC and two types of IC and reported no significant differences between SEC and IC animals with respect to number of lines crossed over the last three days of open field testing. Clear EC/IC differences did emerge, however. This led them to question "the intuitive view that SEC will cause an accentuated form of post-EC behaviour" (p749). In their study, however, Rose et al (1985a) housed a much smaller number of animals in their SEC than were employed in the present experiment, a design feature which may have influenced their reactivity results. However, they did find significant SEC/IC differences in Skinner box behaviour, suggesting that SEC effects were present although in their experiment these effects were task specific.

As a consequence of this earlier research and the lack of clear SEC effects across all behavioural measures, in this thesis it was necessary to establish significant differences between the animals raised in the present superenriched environment (SEC) [1], and the impoverished condition (IC),

[1] In the present SEC it should be noted that four times as many animals were housed together in the environment than were employed by Rose et al (1985a).

before employing these environments as maternal manipulations [2]. To distinguish the effects of SEC from the effects of IC, following Rosenzweig and Bennett and Diamond's recommendation (1972) a third and control environment the standard condition (SC) was also included in the design. If significant differences emerged between SEC and IC animals, comparison with SC animals would allow the contribution of SEC and IC to the effects to be assessed. Other than changing the nature of the enriched environment, for ease of comparison it was decided that all other methodological features would be kept the same as those typically employed in the enrichment literature. Therefore, animals were bought in directly from the supplier (F0 generation) and SEC, SC and IC experience was kept to 30 days.

Apart from establishing that the SEC employed in the present thesis produced animals that were significantly different from those reared in isolation, as mentioned earlier, it was also necessary to provide a behavioural profile of these animals against which to compare their future offspring. As both male and female offspring were to be investigated, both male and female animals were employed in the present work. Consequently, in this first experiment three tests, that not only cover a wide range of behavioural measures but which have commonly been used in the EC/IC literature and which have demonstrated clear EC/IC differences in Hooded Lister rats (Lamden 1985; Curry 1987) were included in the design, namely the open field, the Skinner box and the visual cliff [3].

5:2:2 METHODOLOGY

a) Subjects:

These were 30 male and 30 female F0 generation Hooded Lister rats, bought from Olac Harlan Ltd. at weaning age (19-21 days) and assigned in equal numbers to SEC, SC and IC for 30 days.

[2] Furthermore, although it could be argued that a more traditional enriched environment (EC) should also have been included in this experiment for comparison purposes, the use of EC has been extensively reviewed in chapter two of this thesis and was deemed a sufficient literature against which to match the SEC animals' behavioural profile.

[3] For a fuller profile of EC/IC behaviours in these experimental tests the reader is referred to chapter two.

In order to maximise the SEC experience, a further 35 "padding" animals were included in the SEC environments. Male and female animals were housed separately. At the end of this period, the animals were weighed and housed in individual laboratory cages and coded by a technician so that the experimenter was unaware of each animal's background.

b) Environments and Apparatus:

The SEC, SC and IC environments employed in this experiment, as well as the open field, the visual cliff and the Type II Skinner box system are all detailed in the general methodology chapter (Chapter 4).

c) Procedure:

On removal from the differential environments, animals were maintained in individual cages [4] for one week prior to the start of testing. This delay has been employed elsewhere in the literature (Rose, Dell and Love 1985a; Rose, Love and Dell 1986; Rose, Dell and Love 1987), and is intended to allow the animals to habituate to both their new housing and being handled prior to the start of testing, so that results are not confounded by working with unduly stressed animals. Animals were then tested following the procedure outlined in Table 5:1.

DAYS	APPARATUS	TRIALS PER DAY
1 to 5	Open Field	One
6 and 7	None	None
8 to 13	Skinner Box	One
14 to 17	None	None
18	Visual Cliff	Two

Table 5:1 Order of testing of animals employed in this experiment.

[4] Although housing animals individually effectively isolates them prior to testing, this procedure does not appear to alter the typical EC-IC behavioural effects noted in the literature (Lamden 1985).

Following five days of open field testing, on Day 6, animals were placed on a 12 hour food deprivation schedule (Rose, Dell and Love 1985a) in readiness for the operant training starting on Day 8. Animals were then maintained on a deprivation schedule until the end of testing on Day 13, when they were returned to an ad libitum diet. Visual cliff testing started after four days of normal maintenance. Procedures for the specific behavioural tests are given below:

OPEN FIELD PROCEDURE: On each of the five testing days, animals were weighed and taken to a short-term holding area, from which individual animals were removed and carried into the experimental room containing the open field. Each trial lasted three minutes and the rat was placed next to the wall facing the centre of the field. Measures of ambulation, rearing and time spent in the centre of the field were recorded by the experimenter in the observation room. At the end of each trial, the rat was returned to its home cage, number of fecal boli counted and the open field wiped clean and disinfected with Tego spray. The procedure was then repeated with the next subject. Animals were run in a different random order on consecutive days starting at 10am and continuing until all the animals had been tested. During this time, animals were maintained on an ad libitum diet.

SKINNER BOX PROCEDURE: Skinner box testing started on Day 8, after the animals had been on a deprivation schedule for two days. Subjects were weighed at the beginning of each day of testing and then taken to a short-term holding area until required. Subjects received one 30 minute Skinner box trial on each of six consecutive days. Reinforcement consisted of one pellet of food paired with one second of illumination of the four house lights, similar to the rapid training procedure employed by Rose, Dell and Love (1985a). The following training schedule was employed: Days 1 and 2-CRF; Days 3 and 4-FR3; Day 5-FR6 and Day 6-FR9, during which time number of bar presses and reinforcements were recorded. As with the open field, testing began at 10am and animals were tested in a different random order on each of the six days.

VISUAL CLIFF PROCEDURE: After having been weighed, subjects were taken to a short-term holding area, in readiness for testing. Each subject received two trials, one with the cliff side

of the apparatus set at 1 inch below the glass, the other with the cliff set at 12 inches below the glass. Order of trial presentation was determined randomly, as was position of experimenter relevant to the apparatus. Having set the movable shelf at the approriate distance from the glass, a trial consisted of removing the animal from its home cage, placing it on the barrier separating the shallow and deep sides of the cliff so that it faced away from the experimenter and recording side chosen [5] and latency to descend onto the glass. Descent was counted as having occurred with the animal placing one front paw on the glass. Testing started at 10am and continued until all the animals had received their two trials. As before, animal running order was randomised. Following this test, animals' labels were recoded so that the experimenter now knew which group each animal came from and analyses were performed on the data.

5:2:3 RESULTS

Taken overall, the results of the three behavioural tests demonstrate differences between SEC, SC and IC animals, qualified by the sex of the animals and are generally comparable to previous findings in the literature. In this section, results will be discussed in detail in relation to each piece of apparatus.

a) Open Field:

Analysis of variance of the lines crossed measure revealed significant differences between the three environmental groups $F(2,54)=3.40$ $p<0.03$, and days, $F(4,216)=4.84$ $p<0.001$, these main effects being qualified by a significant days by environment interaction $F(8,216)=7.51$ $p<0.001$. As can be seen from Figure 5:1, which shows the numbers of lines crossed over the five days of testing, exposure to SEC, SC and IC produces differential patterns of responding, such that SEC and SC groups gradually reduce their activity over days, whilst IC's maintain higher levels of responding. Post hoc analysis, using the Newman Keuls test, confirmed that SEC and SC

[5] Labelled "Shallow" or "Deep" irrespective of the real depth of the "Deep" side.

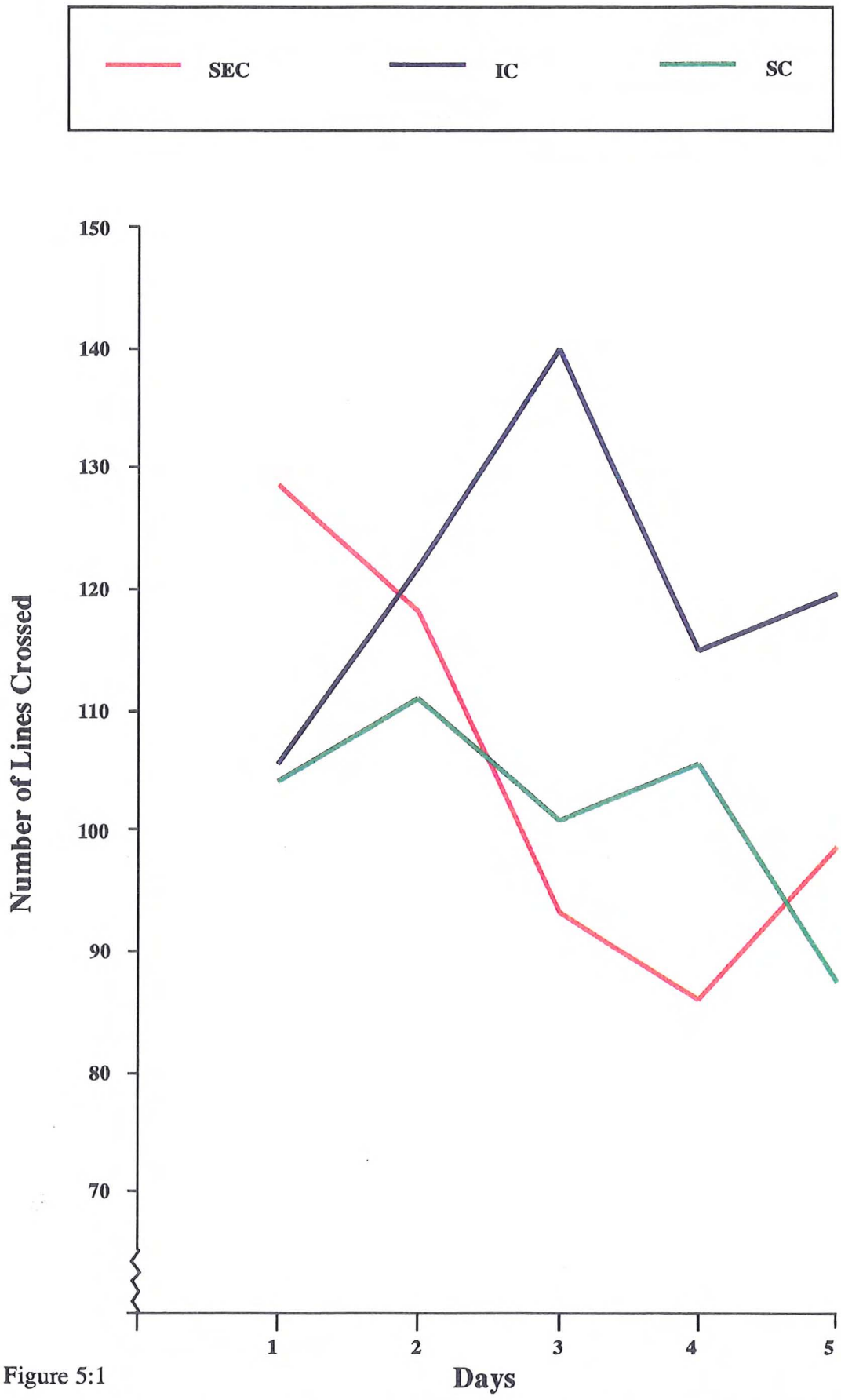


Figure 5:1

Mean number of lines crossed by animals exposed to SEC, SC and IC over the five days of open field testing, male and female groups' scores combined.

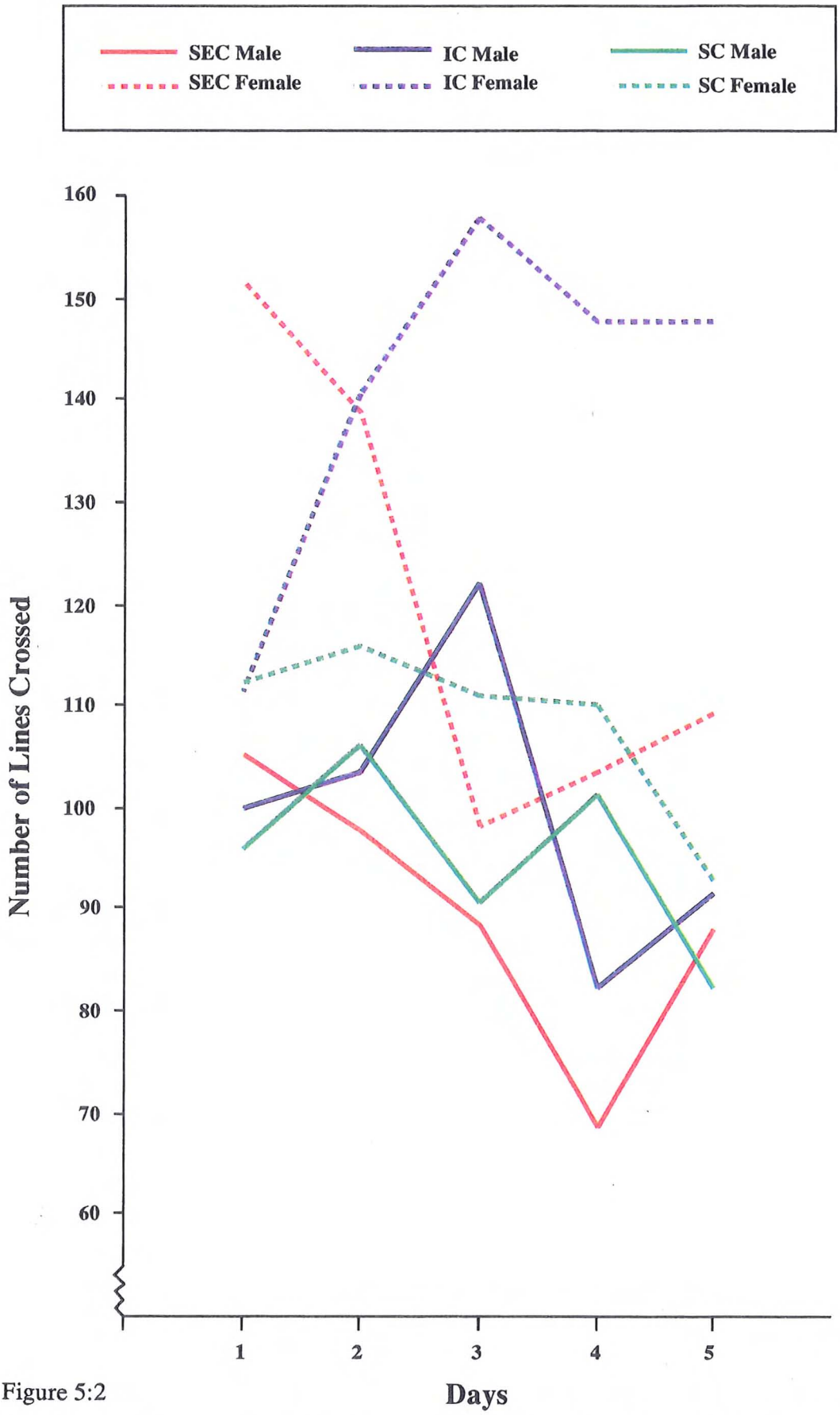


Figure 5:2

Mean number of lines crossed by male and female animals exposed to SEC, SC and IC over the five days of open field testing

animals were significantly different from IC animals ($p<0.05$) but not significantly different from each other over the whole period (Means of the groups: SEC 104.0; SC 101.47; IC 120.47).

As can be seen from Figure 5:1, on Day 1, which has been found to factor load on emotionality (Whimbey and Denenberg 1967b) and has been considered as a measure of exploratory behaviour (Hayes 1960) SEC animals were more active than either their SC or IC counterparts. This was confirmed by post hoc Newman Keuls tests, $p<0.01$. In addition, the ANOVA over the five days of testing also revealed significant sex differences. As can be seen from Figure 5:2, females were more active than males $F(1,54)=20.75$ $p<0.001$. There was also a days by environment by sex interaction $F(8,216)=2.65$ $p<0.05$ which warranted further analysis. Post hoc Newman Keuls tests were performed on the six groups' performances totalled over the five days of testing. Considering the male animals first, although overall IC animals crossed more lines than either their SC or SEC counterparts, these comparisons were not significant. For the female groups, however, IC females were found to cross more lines than their SEC ($p<0.10$) and SC counterparts ($p<0.01$). Furthermore, both the SEC and IC females were more active than the SEC males ($p<0.05$ and $p<0.01$ respectively), whilst IC females were also more active than the SC and IC males ($p<0.01$ for both comparisons). These post hoc comparisons are presented in more detail in the appendix.

With respect to the number of rears, again significant differences emerged between the environmental groups $F(2,54)=3.16$ $p<0.05$, with SEC animals rearing more than IC and SC animals (Means of rears over the five days of testing: 13.94, 11.91 and 10.22 respectively). This finding was qualified by a significant days by environment interaction $F(8,216)=6.53$ $p<0.001$ which can be clearly seen in Figure 5:3. SC animals appeared to habituate faster than both the SEC and IC groups. As with the lines crossed measure, significant sex differences emerged $F(1,54)=7.63$ $p<0.05$ in favour of the females (see Figure 5:4). In this analysis, however, no days by sex by environment interaction emerged $F(8,216)=0.66$ $p>0.05$ suggesting that no significant differences in patterns of responding existed between the males and females from the three environment

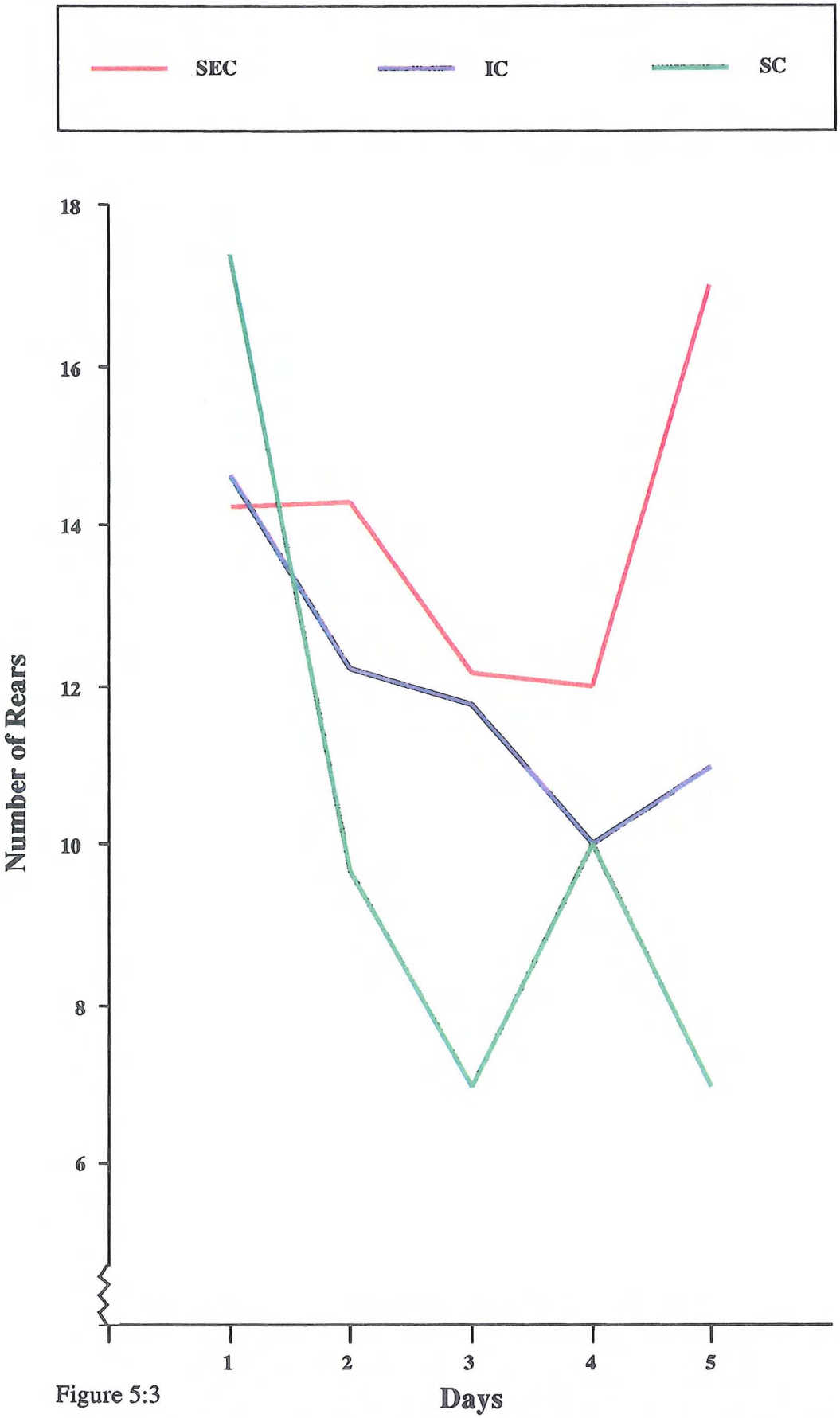


Figure 5:3

Mean number of rears by the SEC, SC and IC groups over the five days of open field testing, male and female groups' scores combined.

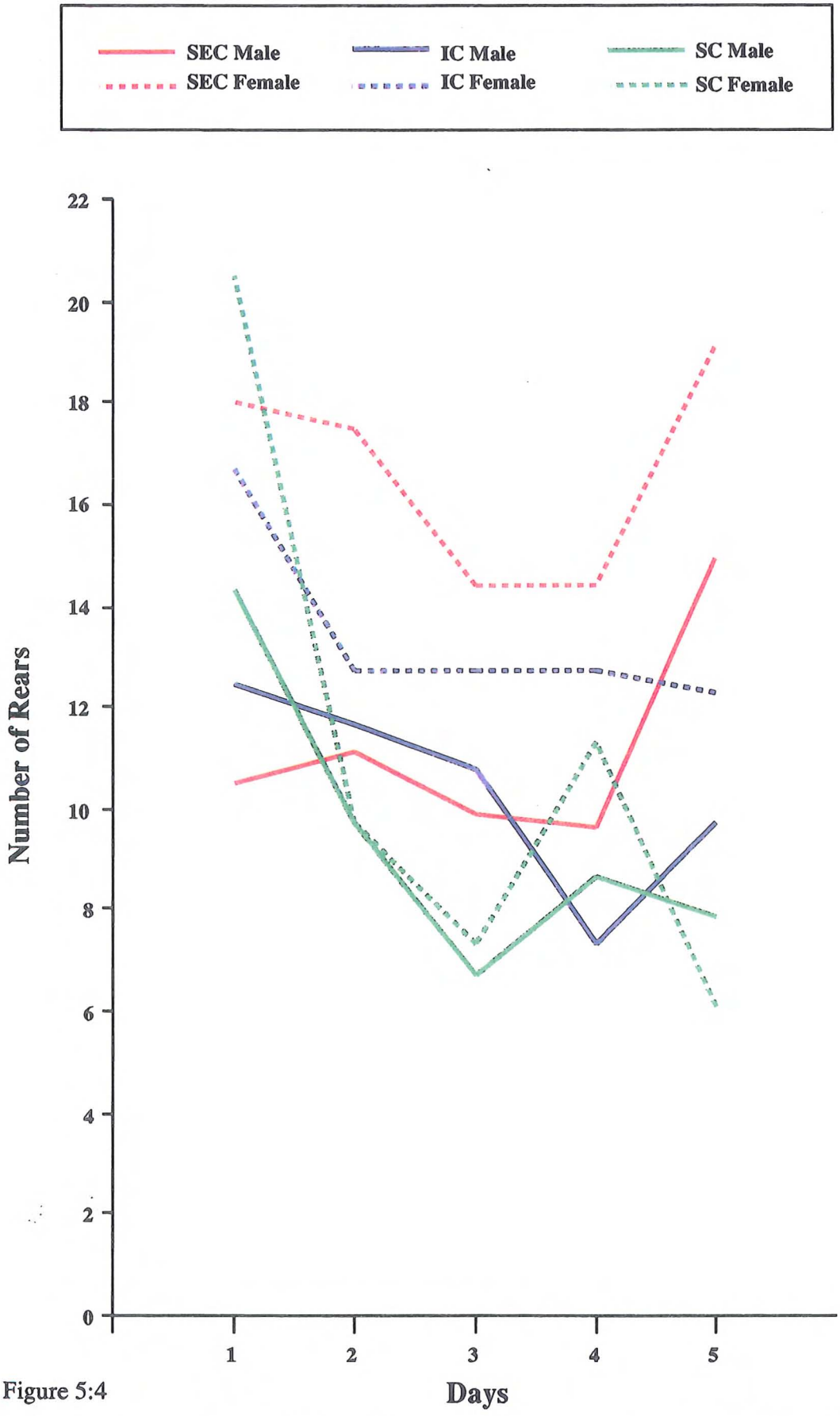


Figure 5:4

Mean number of rears by male and female animals exposed to SEC, SC and IC over the five days of open field testing.

groups, consequently no further analyses were performed on these data.

As would be expected from Figures 5:3 and 5:4, there was also a significant days effect $F(4,216)=12.48$ $p<0.001$ and a significant days by sex interaction $F(4.216)=2.34$ $p<0.05$ females rearing more over days than males. No other significant interactions emerged in this analysis.

Of the remaining two dependant variables, time spent in the centre of the open field and number of defecations, no significant differences emerged between the experimental groups in either ANOVA, $F(2,54)=1.47$ $p>0.05$, and $F(2,54)=1.72$ $p>0.05$ respectively. Furthermore, no sex differences emerged with respect to the time in centre measure $F(1,54)=0.80$ $p>0.05$. However, males were found to defecate more than females $F(1,54)=11.33$ $p<0.001$ and over days, SEC, SC and IC males amounts of defecation varied such that over days SC males increased their amount of defecation compared with the other two groups $F(8,216)=2.51$ $p<0.01$. For means, please see the appendix.

b) Skinner Box:

Analysis of variance of the number of bar presses over the six days of training revealed no overall differences between the three environmental groups $F(2,54)=2.13$ $p>0.05$, although there was a significant main effect due to sex $F(1,54)=15.46$ $p<0.001$, males bar pressing more than females. This can be seen more clearly from Figure 5:5, which details the learning curves of the six experimental groups. As would be predicted in a learning experiment, there was also a highly significant days effect $F(5,270)=94.99$ $p<0.001$, qualified by a days by sex interaction $F(5,270)=9.65$ $p<0.001$ reflecting the sharper learning curves of the male offspring. Of particular interest to the present study, however, was the final significant interaction, days by sex by environment $F(10,270)=2.30$ $p<0.01$. This suggested that the six offspring groups' behavioural patterns over the six days were different from each other, which is evident from Figure 5:5. To explore this interaction further, post hoc Newman Keuls comparisons of the six groups' performances on the last day of Skinner box testing were carried out. Considering the male animals first, as would be

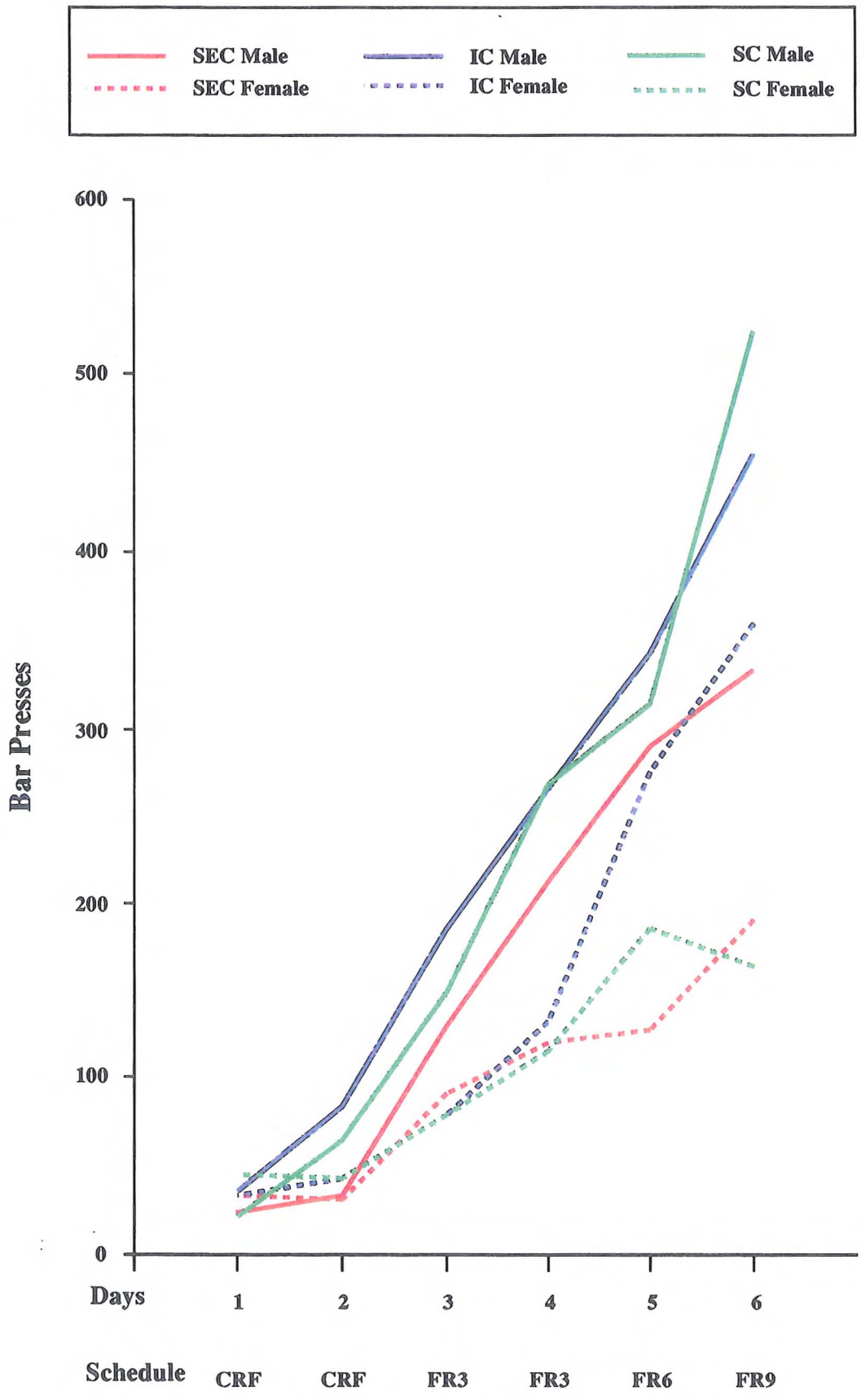


Figure 5:5

Mean number of bar presses by the male and female groups exposed to SEC, SC and IC over the six days of Skinner box training.

expected from the graph (Figure 5:5), SC and IC animals with higher rates of bar pressing were significantly different from the SEC animals, $p< 0.01$ and $p<0.05$ respectively, but not different from each other ($p>0.05$). With the female groups, however, a slightly different pattern emerged. As with the males, female IC animals bar pressed significantly more than their SEC counterparts ($p<0.01$), but unlike the males, IC females also bar pressed more than their SC counterparts ($p<0.01$). SC and SEC females did not differ from each other ($p>0.05$). In addition, SEC males bar pressed more than both the SEC and SC female groups ($p<0.01$ for both comparisons), IC males bar pressed more than the SEC and SC female groups ($p<0.01$ for both comparisons) and the group with the highest rate of bar presses, the SC males, bar pressed more than all three female groups ($p<0.01$ for all three comparisons).

c) Visual Cliff:

Table 5:2 details the number of animals choosing deep and shallow sides in the two visual cliff trials, when the movable shelf was set at either 12 inches or 1 inch below the glass. Although there appears to be no real difference in choices between the groups when the deep side was set at 1 inch, animals choosing the shallow side only marginally more than the "deep" side, when the deep side was set at 12 inches, the profile changes somewhat. Both SC and IC animals chose the shallow side in most cases, whilst SEC animals were less influenced by the apparent depth of the "deep" side. Chi squared analyses for k independent samples on each of the trials, however, did not reveal any statistically significant differences between the three groups, $\chi^2=0.13714$ and 3.73159 $p>0.05$ for the one and twelve inch trials respectively.

	SEC(1)	IC(1)	SC(1)	SEC(12)	IC(12)	SC(12)
SHALLOW	12 (60)	11 (55)	12 (60)	13 (65)	18 (90)	16 (80)
DEEP	8 (40)	9 (45)	8 (40)	7 (35)	2 (10)	4 (20)

Table 5:2 Numbers of animals (expressed as percentages in brackets) choosing deep and shallow sides of the visual cliff, when the "deep" side was set at either 1 inch or 12 inches.

ANOVA of the latency to descend onto the cliff revealed a significant difference between the experimental groups $F(2,54)=5.23$ $p<0.001$. Post hoc comparisons, using the Newman Keuls test, revealed the significance to lie between the SEC and IC ($p<0.05$) and SEC and SC ($p<0.01$) groups. No differences emerging between the IC and SC groups. SEC animals took less time to descend onto the cliff (5.2 seconds) than the other two groups (IC: 11.72 seconds, SC: 15 seconds). None of the other main effects was significant, nor were there any significant interactions, $F(1,54)=0.22$ and 0.29 $p>0.05$ for sex and trials respectively, $F(2,54)=0.80$ $p>0.05$ for the sex by group interaction.

d) Summary of Significant Main Effects:

In the open field, significant differences emerged between the three experimental groups' patterns of responding such that IC animals and in particular, the female IC animals, maintained higher levels of responding than their SEC and SC counterparts over days. With the rearing measure, although SEC animals reared more than IC and SC animals, group profiles over days also varied, SC animals' number of rears reducing at a faster rate over the first three days than their SEC and IC counterparts, SEC animals increasing their number of rears on Day 5. With the Skinner box procedure, differences emerged between the both the male and female IC and SEC groups, the latter maintaining lower bar press rates over all days. SC animals' bar press reponses, however, were less consistent and were related to the sex of the animal. Finally, latency to descend onto the visual cliff was measured, as was side chosen. SEC animals took less time to descend onto

the apparatus than their SC and IC counterparts and were more likely to pick the deep side especially when it was set at 12 inches, than either of the other two groups, although this latter result was not statistically significant.

5:2:4 DISCUSSION

The present experiment had two main objectives: firstly, to see whether the enriched condition employed in this thesis, which was adapted for longer term use, would still yield animals whose behavioural profile was significantly different from their IC counterparts and secondly, to provide a behavioural profile of both male and female animals against which to compare male and female offspring. Both of these aims were achieved.

Considering first the efficacy of the present enriched environment, clear SEC/IC differences have emerged in all three behavioural tasks, albeit qualified by sex of animals. Furthermore, in most instances, these differences are similar to those typically found in studies employing the more traditional EC. The nature of these differences will become apparent as the findings of this experiment are considered in some detail in the following paragraphs.

Starting with the open field results, in the present research, IC animals and in particular female IC animals, maintained consistently higher levels of activity over the last three test days, than did their SEC counterparts [6]. This pattern of increased IC ambulation has been reported elsewhere in the literature (Woods et al 1960; Levitsky and Barnes 1972; Fessler and Beatty 1976; Domjan et al 1977) and has commonly been found in the present author's laboratory (Lamden 1985; Curry 1987; Dell and Rose 1987) [7]. Moreover, IC animals in the present experiment were more active than their SC counterparts (although this was only statistically significant in the female animals), suggesting that ambulation differences are due to differential IC response patterns, rather than to those of the SEC animals. This IC/SC difference is consistent with work by Syme

[6] In the open field significant differences in the lines crossed measures only emerged between the female groups. The importance of these sex differences are highlighted later on in this discussion.

[7] Interestingly, this pattern is typically found in male animals in the author's laboratory, unlike the present research where the significant differences only emerged in the female animals.

(1973), Einon, Morgan and Sahakian (1975), Morgan and Einon (1976), Einon, Morgan and Kibbler (1978) and Einon and Morgan (1978), all of whom have reported their IC animals to be more active than socially housed animals.

With respect to the rearing measure, analysis of variance of both male and female groups together revealed significant group effects, SEC animals rearing more than their SC and IC counterparts. As rearing behaviour has been interpreted elsewhere as a measure of exploration (Dell and Rose 1986), this finding, coupled with the higher levels of SEC ambulation on the first day of open field testing, suggests that these animals are either initially more reactive [8] or more exploratory than their SC and IC counterparts. The fact that no overall significant differences emerged between the groups with respect to either the defecation or time in centre measures, suggests a lack of differences in emotionality between the groups. This in itself is unusual, as IC animals have often been considered more emotional than their EC counterparts (Ader and Friedman 1964; Moyer and Korn 1965). However, if emotionality is taken to encompass timidity, or reaction to novelty rather than the more extreme construct of fear, then these results, taken with those of the visual cliff test, in which IC animals were more reluctant to descend onto the cliff, begin to make sense.

Finally, in the open field data, clear sex differences emerged, with females demonstrating higher levels of activity as measured by number of lines crossed, and exploration as measured by number of rears, than males. With respect to the defecation levels, however, the obverse was true, the higher number of male fecal boli suggesting either a greater emotionality in these animals (Whimbey and Denenberg 1967b) or more pragmatically a greater food intake (Archer 1973). Of particular interest to the present work, however, was the significant sex by days by environment interaction in activity as measured by number of lines crossed, which when explored further by post hoc analysis, revealed that the SEC/SC/IC patterns of responding were only statistically significant in the female groups [9]. This finding parallels Woods et al's (1960) report of a larger EC-

[8] This is only a tentative suggestion, as there are certain questions that the notion of differential reactivity does not necessarily explain. For example, if the animals do differ in reactivity, why is it that SEC animals only cross more lines on day one, whilst rearing more throughout?

[9] It should be emphasised at this point that for all the open field measures, the relative position of the SC group with respect to the SEC and IC groups is the same for male and female animals. This is not the case in the Skinner box study, a point that will be addressed further on in this discussion.

IC difference in Hebb-Williams performance for females, but as Renner and Rosenzweig (1987) remark "the exact character and potential functional significance of this dimorphism remains unclear" (p63). Furthermore, the lack of statistical significance between the males in the lines crossed measure in this study is consistent with the work of Rose et al (1985a), who found no significant differences between their male SEC and IC animals, suggesting that with this more complex type of environment, sex of animal is an important factor in activity measures, a point also noted by Archer (1973).

Why significant SEC/SC versus IC effects in number of lines crossed should emerge in female animals, but not in their male counterparts is not clear at the present time. Looking at Figure 5:2, however, it appears that the SEC males' performance is similar to that more normally found in EC males, namely lower levels of activity and reducing activity over days when compared with their IC counterparts (Lamden 1985). Furthermore, the SC group's performance is not dissimilar to that of previous studies, that is gradually reducing activity as measured by lines crossed, over days. In the present study, however, IC males reduced their lines crossed behaviour over the last few days rather than maintaining the higher levels of activity that have been found before in the author's laboratory (Rose et al 1985a) using the same strain of rats and type of impoverishment. So, whatever the reason, the lack of differences between the SEC, SC and IC males is more to do with the IC males' performance than their SEC and SC counterparts. This in itself is important, as one of the purposes of this study was to ascertain whether or not the SEC employed in this thesis produces animals with behavioural patterns that resemble those found in animals reared in the more traditional enriched environment based on the EC used by Rosenzweig and his colleagues at Berkeley. From the behavioural patterns of both male and female SEC animals in the lines crossed and other measures, the use of the SEC in this thesis appears to have been justified.

Moving on to the Skinner box data, again clear SEC/IC differences, emerged with both male and female IC animals bar pressing more than their SEC counterparts. This parallels the work

of Rose, Dell and Love (1985a) in which both male SC and IC animals were found to bar press significantly more than their SEC and EC counterparts. The higher level of responding noted in the IC animals in this present experiment is not unusual. In simple operant procedures such as the one employed in the present research, several authors have reported higher levels of IC bar pressing (Coburn and Tarte 1976; Lamden and Rose 1979; Joseph and Gallagher 1980; Nau, Elias and Bell 1981; Rose and Lamden 1983; Rose, Dell and Love 1985a; Rose, Love and Dell 1986; Rose, Dell and Love 1987; Curry 1987). One explanation that has been advanced to account for this phenomenon is that IC animals are stimulus-seeking (Lamden and Rose 1979; Chadha and Rose 1981) following their early partial sensory deprivation [10]. What is relevant to the present thesis however, is that the SEC animals perform differently from IC animals and in a manner similar to subjects reared in the more traditional enriched condition. Finally, in the Skinner box procedure, the behaviour of SC animals relative to their SEC and IC counterparts differed according to sex, male SC animals paralleling IC behaviour, whilst female SC animals followed SEC patterns of responding. Why this should have occurred, is at present unknown.

In the last apparatus to be employed, the visual cliff, again evidence of SEC/IC differences emerged. Considering first the side chosen measure, although not statistically significant, there was a tendency for SEC animals to descend onto the deep side of the cliff, when the cliff was set at 12 inches, more often than IC animals. This finding is somewhat counterintuitive, in that several reports in the literature have suggested EC animals employ distance cues more efficiently than IC subjects (Hymovitch 1952; Forgays and Forgays 1952; McCall et al 1969). Indeed, as Lamden (1985) has suggested, the nature of the EC environment must afford its occupants greater experience of depth than that encountered by either SC or IC animals. This appears to be borne out in her research and in that of Eichengreen et al (1966), where EC animals were found to have a more highly developed depth perception. More recently, however, Curry (1987) has failed to replicate this earlier work, reporting no significant differences between his EC and IC groups, in terms of side chosen. Furthermore, although Lamden reported increased latencies

[10] In particular, this hypothesis suggests that IC animals are bar pressing to maximise the sensory stimulation afforded by a composite reinforcer of light and food, such as was employed in the present work.

to descend in her EC animals, a finding she interpreted as reflecting greater decision making in these animals, Curry's work, similar to the present research, found the opposite, namely that his IC animals took longer to descend onto the cliff, than his EC subjects.

This decreased latency to descend in EC (Curry 1987) and SEC (present work) animals may well reflect procedural differences when compared with Lamden's earlier work. In her experiment, significant differences in favour of her EC animals only emerged across trials, after several days of testing. Both the present work and the procedure employed by Curry, tested animals on one day alone. Latency to descend and side chosen in the present experiment may well therefore reflect SEC and IC animals responses to a novel apparatus, rather than depth perception per se. Indeed, considering the literature investigating the latency to emerge into a novel environment, isolates have been found to take significantly longer to emerge than their socially housed counterparts (Ader and Friedman 1964; Gill et al 1966; Konrad and Bagshaw 1970; Morgan 1973; Einon and Tye 1975; Benton and Brain 1981). Perhaps in the present work, SEC animals were keen to explore and descended quickly onto the cliff, without much concern for the apparent danger of a 12 inch drop [11]. IC's on the other hand, may well have been more cautious taking their time to descend onto the apparatus and ensuring that they chose the appropriate side. This rationale is further substantiated by the performance of the SC group, who like the IC's would have had little experience of depth perception in their environment. Although less cautious (though not significantly so) than IC animals in terms of latency to descend, these animals were significantly slower than the SEC animals and more likely to chose the shallow side.

In summary, therefore, the results of this experiment demonstrate that the SEC employed in the present work produces animals which are very different from IC subjects and whose behavioural profiles are similar to those of animals housed in the more traditional enriched environments typically found in the literature. This is particularly true of female animals which is notewothy, as they will be providing the environmental influences to be passed on to the next generation. In

[11] Indeed, given that the SEC animals regularly launched themselves off walls onto the floor of their enriched cage, it is unlikely that falling a foot or so holds much fear.

particular, SEC animals were more exploratory, but their activity patterns suggested that they habituated quickly, whilst IC animals were more cautious to start with, but once embarked on an activity, perseverated in that behaviour. In addition, IC animals appeared to seek stimuli, something that the SEC animal was less concerned with. SC animals were more like the SEC with respect to novelty, but appeared also to respond in such a way as to maximise stimuli [12].

5:3 EXPERIMENT TWO

5:3:1 INTRODUCTION

In the preceding experiment behavioural differences were found between animals exposed for *thirty* days to SEC, SC and IC. In the methodology to be employed in the present thesis, however, females exposed to the differential environments as a maternal manipulation prior to pregnancy, were to be maintained in their respective environments until sexually mature, that is for sixty three days. This length of exposure, although not atypical in the literature investigating the effects of enrichment on anatomical and biochemical brain changes (Rosenzweig, Bennett and Diamond 1972a; 1972c), is less commonly employed by those researchers interested in the behavioural consequences of differential environments. Therefore, it was considered appropriate to investigate whether the increase in duration of exposure to the SEC employed in the present thesis would have a different affect on the animals' behaviour than shorter exposures.

A second and related issue concerns the effects of pregnancy itself on animals exposed to differential environments. A chance finding by Diamond, Johnson and Ingham (1971) reported that the occipital cortex depth differences found between non-pregnant EC and IC female rats were no longer apparent post partum. Further research confirmed this unexpected result and attributed the pregnancy induced alterations in the normal EC/IC pattern to an increase in the cerebral cortical depth of the pregnant impoverished animals (Hamilton, Diamond, Johnson and

[12] The relative contributions of SEC and IC to the patterns of behaviour are discussed more fully in chapter nine.

Ingham 1977). Mediation of this change has been tentatively put down to hormonal influences, preliminary evidence coming from work with synthetic hormones (Hoover and Diamond 1976). Given that the present thesis is concerned with the effects of differential maternal experience on the offspring of animals exposed to SEC, SC and IC, if pregnancy can change cortical depth and alter or mask the response to the environment in the IC condition, then the use of this environment as a maternal manipulation might well be invalidated. Quite simply, if pregnancy and/or the period that the animals are individually housed rather than being maintained in their respective environments remove any SEC/IC brain differences, then there may be no behavioural differences in the dams, nor, in turn, in the offspring themselves.

One way to test for this pregnancy masking effect was to quite simply investigate the behaviour of differentially housed females post partum. Consequently the present experiment was designed to investigate the effects of environmental enrichment and impoverishment prior to pregnancy on the behaviour of female rats post partum. Unlike the previous experiment, no attempt was made in the present work to provide a detailed behavioural *profile* of these animals, consequently only one behavioural test was employed, namely the open field, chosen because it provides one of the most reliable measures of EC/IC differences.

5:3:2 METHODOLOGY

a) Subjects:

These were 27 female F1 generation Hooded Lister rats, bred in the laboratory at Goldsmiths' College, from F0 generation dams bought in from Harlan Olac Ltd. Assigned in equal numbers, using a split litter technique, to either SEC [13], SC or IC at weaning age (19-21 days), these animals were maintained in differential environments until sexually mature, after which they were mated and placed into individual parturition cages (following the breeding procedure outlined in

[13] As in experiment one, this environment contained additional "padding" animals, as did the SC environment, when required.

Chapter 4). After the arrival of their litters, the subjects were left undisturbed until their pups were weaned at 19-21 days. They were then weighed and placed into individual laboratory cages.

b) Environments and Apparatus:

The SEC, SC and IC environments employed in this experiment, as well as the open field apparatus, are detailed in Chapter 4.

c) Procedure:

On removal from their litters, subjects were maintained in individual cages for seven days to allow lactation to cease, prior to testing [14]. During this time, all the animals were recoded by a technician so that their experiential background was unknown to the experimenter, thus removing any possibility of experimenter bias in recording results (Rosenthall 1966). On Day 8, subjects commenced testing in the open field, with running order randomised at the start of each day. The procedure followed in this experiment was the same as in experiment one and comprised one three-minute trial on each of five consecutive days. During this time animals were maintained on an ad libitum diet. Number of lines crossed, rears, defecations and time spent in the centre circle were recorded.

[14] It should be noted that Diamond et al (1971) sacrificed their animals directly parturition had occured

5:3:3 RESULTS

a) Open Field:

Analyses of variance performed on each dependant measure over the five days of testing revealed highly significant differences between the three post partum groups.

Considering first the number of lines crossed, significant environment $F(2,24)=15.53$ $p<0.001$ and days $F(4,96)=2.37$ $p<0.05$ main effects emerged qualified by a significant environment by days interaction $F(8,96)=2.54$ $p<0.01$. As can be seen from Figure 5:6, which shows the numbers of lines crossed over the five days of testing, exposure to SEC, SC and IC prior to pregnancy produced animals with significantly different patterns of responding post partum. Specifically, over the five days, both SC and IC groups maintained higher levels of activity than the SEC animals. Furthermore, post hoc analysis using the Newman Keuls test revealed significant differences between SEC and IC groups ($p<0.01$) and SEC and SC groups ($p<0.01$), but no differences between SC and IC groups. Overall, IC and SC groups crossed more lines than did the SEC subjects.

As can be seen from Figure 5:7 detailing the animals' rearing performance over the five days, a similar pattern to that found in the lines crossed measure also emerged, namely that IC and SC groups reared more than their SEC littermates. This was confirmed statistically using ANOVA $F(2,24)=12.75$ $p<0.001$ and the Newman Keuls test (SEC vs IC $p<0.01$; SEC vs SC $p<0.01$; SC vs IC not significant $p>0.05$). Unlike the lines crossed measure, however, no significant days main effects emerged $F(4,96)=1.80$ $p>0.05$, nor was there a significant days by environment interaction $F(8,96)=0.94$ $p>0.05$.

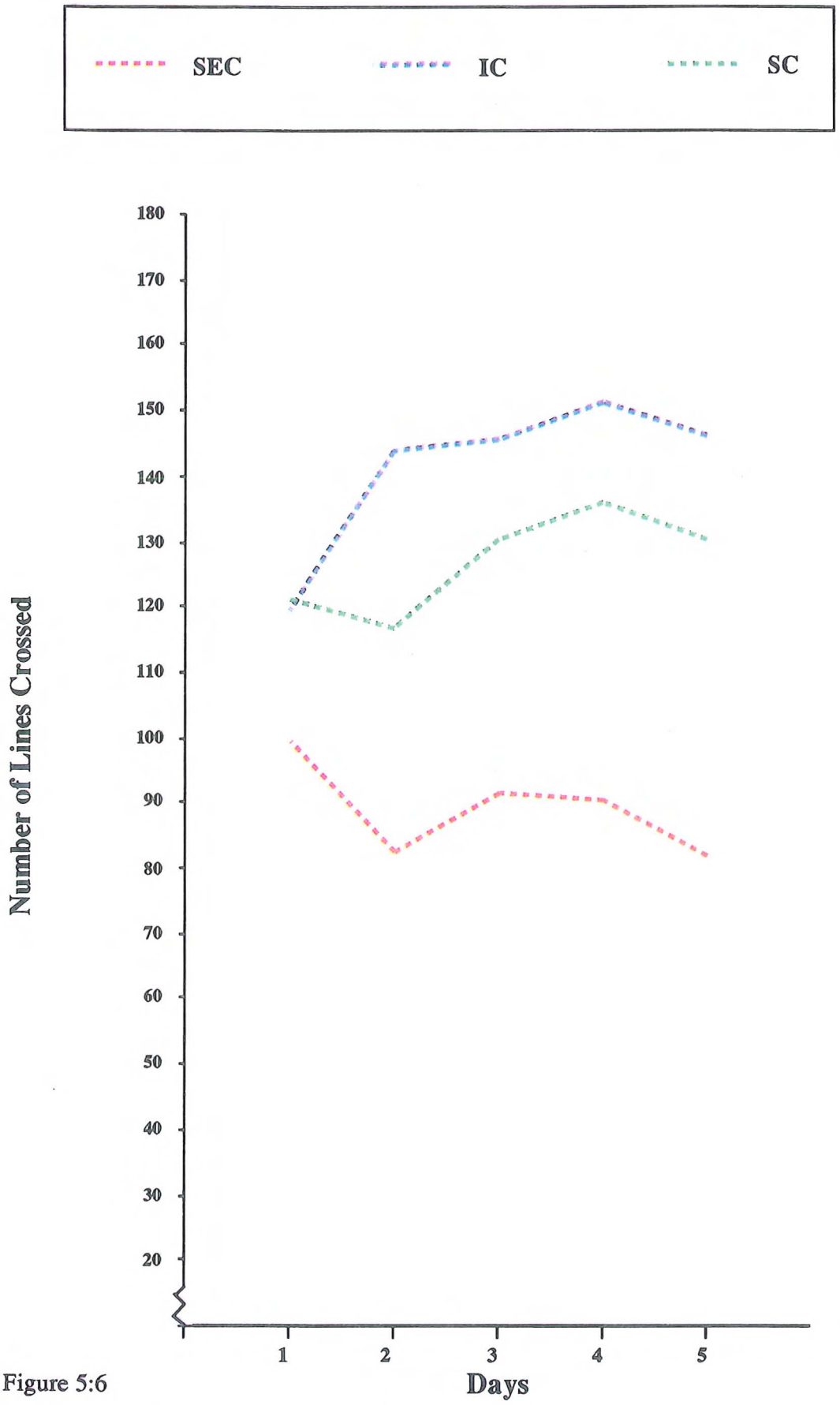


Figure 5:6

Mean number of lines crossed by the postpartum females, exposed to SEC SC and IC prior to pregnancy, over the five days of open field testing.

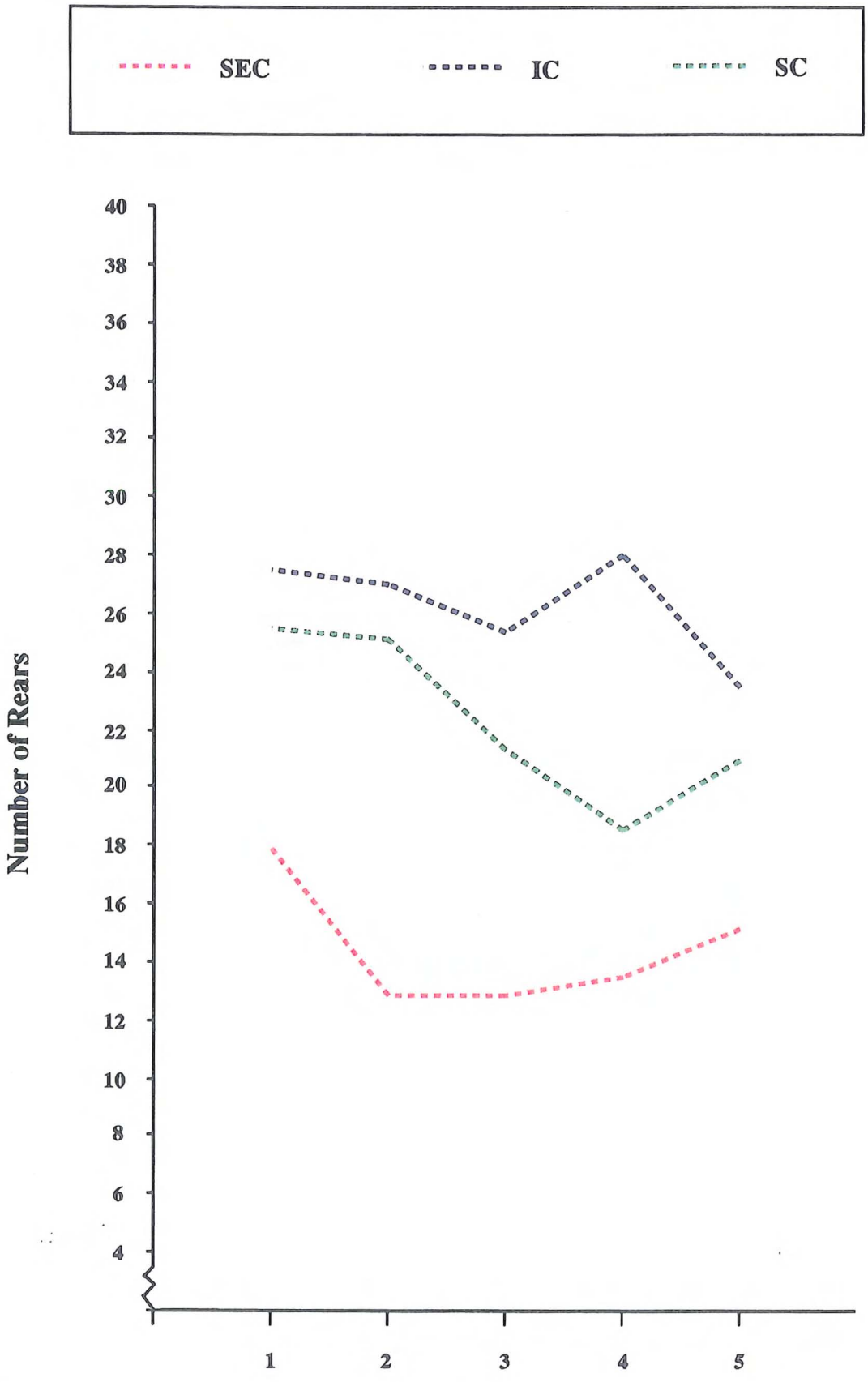


Figure 5:7

Mean number of rears by the postpartum females, exposed to SEC, SC and IC prior to pregnancy, over the five days of open field testing.

As in this experiment none of the animals defecated whilst in the experimental apparatus, the final measure to be analysed was the number of seconds spent in the centre of the open field, which revealed one significant main effect. Animals raised in the SEC spent significantly less time in the centre than their IC littermates $F(2,24)=3.29$ $p<0.05$, post hoc analysis $p<0.05$. No other differences emerged between the groups (Means: IC:7.89; SC:6.31; EC:4.73), $F(4,96)=2.12$ and $F(8,96)=1.86$ $p>0.05$ for days and days by environment respectively).

5:3:4 DISCUSSION

The purpose of this experiment was to ascertain whether the effects of nine weeks of environmental experience followed by pregnancy and successful rearing of litters removed the behavioural differences typically observed between animals raised in differential environments with no experience of pregnancy, such as the SEC/IC animals in experiment one. Obviously, as outlined in the introduction, if the behavioural effects of differential environments were to be removed in the maternal generation following this procedure, then there would be less reason to hypothesise that any offspring effects would be induced either.

However, the results of this present work clearly demonstrate that activity differences in SEC, SC and IC females are present when the animals are tested post partum and at a time when the difference between the groups in terms of one of the most prominent anatomical brain changes is reported to have disappeared (Diamond, Johnson and Ingham 1971).

Considering this latter point first, before moving on to describe the present results in more detail, Diamond et al's work is unusual in the EC/IC literature and warrants further discussion. Typically, maintaining rats in enriched environments has been found to cause anatomical and biochemical brain changes when compared with socially housed or isolated controls (Rosenzweig, Bennett and Diamond 1972a; 1972c), but the precise causes of these changes, although the subject of much speculation (Rosenzweig and Bennett 1976; Renner and Rosenweig 1987), is less clear. Diamond et al (1971) discovered that the EC/IC differences in cortical depth observable

in non-pregnant female rats were no longer apparent post partum. Moreover, she found that the re-establishment of EC/IC equivalence was due to an increase in cortical depth in IC rats rather than to a decrease in EC counterparts. That pregnancy can interact with environmental stimulation in this way may well help to unravel the mechanisms underlying environmentally induced brain changes.

A second question concerning environmentally induced brain changes relates to their functional significance. Again, although the subject of much speculation (Lamden 1985; Dell and Rose 1986), few firm conclusions have been reached. But here also the pregnancy/environment interaction described above does provide a hitherto unexplored way of investigating any correlation which may exist between brain changes and behaviour in EC and IC animals. To be specific, if environmentally induced changes in cortical depth and behaviour are *causally* related, it would be predicted that differences between EC and IC rats would disappear post partum. The results of the present work demonstrate that this is not the case [15] for one behavioural measure anyway.

Of particular interest to the present work, which is concerned with the transfer of effects across generations, however, was the finding that pregnancy did not remove the SEC, SC and IC behavioural changes noted in experiment one of this chapter. Consequently, these environments can be used as a maternal manipulation. Although procedural differences between experiments one and two (such as length of exposure to the environments and the experience of pregnancy) prevented a statistical comparison of the virgin and post partum animals performance, examination of the means of the groups allow a descriptive comparison to be made.

[15] The present study thus failed to provide any evidence of a correlation between changes in the brain and one of the best documented environmentally induced behavioural changes. Such a finding necessarily calls into question any suggestion that said environmentally induced brain changes play a role in subserving psychological or behavioural function. Moreover, it has serious implications for attempts to utilise environmental enrichment in therapeutic contexts such as combating cognitive deficits due to aging (Rosenzweig 1984) and stimulating recovery of function following damage to the brain (Rose 1988). Firm conclusions, however, would be premature. For example, in the present experiment the animals' behaviour was not examined immediately post partum, whereas in the studies of Diamond and her colleagues (Diamond, Johnson and Ingham 1971; Hoover and Diamond 1976; Hamilton, Diamond, Johnson and Ingham 1977), the brain changes were. Also in her work, Diamond confined analysis to cortical depth changes. Perhaps anatomical investigation at an ultrastructural level may have revealed clear EC/IC differences post partum. It is clear, therefore, that differentially reared female rats must be further investigated post partum both in terms of other brain measures and over a wider range of behaviours before any general statements can be formulated.

MEASURE	GROUP	SEC	SC	IC
Lines Crossed	Virgin	120.34	108.44	140.94
	Post Partum	88.96	126.89	141.29
Rears	Virgin	16.68	10.98	13.42
	Post Partum	14.44	22.29	26.24
Time in Centre	Virgin	1.60	1.40	2.20
	Post Partum	4.73	6.31	7.89

Table 5:3 Mean scores for the virgin and post partum females' open field behaviour, N=10 and N=9 respectively.

Considering first the SEC/IC animals, as can be seen from Table 5:3 in both the virgin and postpartum females, IC animals crossed more lines than did their SEC counterparts. However, this finding was more pronounced in the post partum animals. With respect to the rearing measure, virgin SEC animals reared more than their IC counterparts, whereas for the post partum animals the opposite was true. This suggests that although pregnancy did not remove the behavioural differences between the groups, there were some effects of this experience in the animals. Perhaps pregnancy made the IC animal less emotional and more inclined to explore. This suggestion appears to be born out by the time in centre measure. Although both virgin and post partum IC animals spent more time in the centre of the open field than their SEC littermates, only the post partum comparison achieved statistical significance. Overall, the IC animals, irrespective of whether they have reared litters or not, seem more active than their SEC counterparts. Interestingly, the day one increase in activity in the virgin SEC animals was not apparent post partum, suggesting that these latter rats, with their experiences of pregnancy and rearing of litters are either less emotional than their virgin counterparts or as the rearing measures suggest, less likely to explore. Perhaps the interaction afforded the IC dam by a litter is in itself an "enriching" experience, after all, other than the brief period with the stud male, the postpartum period is the first time these animals would have experienced any form of social contact since being weaned. Furthermore, as the literature which explores the relationship between rat mothers and their offspring has noted, the behaviour of the rat mother has been

found to be modified by her pups (Deis 1968; Grosvenor et al 1970; Mena and Grosvenor 1971; Smotherman et al 1977a; 1977b; 1978). There is no reason to suppose that this might not also be true in the impoverished mother's case.

Finally, considering the relationship of the virgin and post partum SC groups to their respective SEC and IC groups, for the virgin animals, as can be seen from Figure 5:2, SC animals' reduced their number of lines crossed over days as did their SEC counterparts (especially over the first three days of testing) a profile that was very different from that of the IC animals. In the post partum animals, however, SC animals' activity levels were more like that of their IC counterparts (Figure 5:6), suggesting that there may have been an influence on the behaviour of these animals post partum, such that pregnancy served to make them more active. Indeed, this picture is supported by the animals' rearing behaviour, as becomes evident when the number of rears of the post partum groups (Figure 5:7) are compared with those of their virgin counterparts (Figure 5:4). Over days both virgin and post partum SEC animals reared at similar rates, whilst both the SC and IC post partum females doubled their rate of rearing when compared with their virgin counterparts.

In summary, the results of this experiment demonstrate that both exposure to differential environments for nine weeks prior to mating and the additional experience of pregnancy and rearing of litters produce behavioural differences between SEC, SC and IC females justifying the use of these environments as maternal manipulators in the present thesis. However, it should be noted that the behavioural patterns of the virgin SEC, SC and IC animals are not the same as the pattern of differences observed in the post partum SEC, SC and IC animals in the open field, suggesting that pregnancy has an impact on these animals.

5:4 GENERAL DISCUSSION AND CONCLUSION

The purpose of this chapter was to address some practical issues, before embarking on the main focus of the present thesis. In particular, a decision regarding the type of enriched environment

to be used in this research had to be taken and a behavioural baseline of animals exposed to the differential environments employed in this thesis established, against which to compare offspring and grandoffspring behaviours. This was accomplished in the first experiment, in which the "SEC" employed in the present thesis was found to produce animals that were behaviourally different from isolates and of a comparable nature to animals raised in the more traditional Rosenzweig and Bennett "EC". The relative contributions of the different enironments to the behavioural effects is discussed more fully in the final discussion chapter.

Secondly, the methodology employed in the present thesis, namely maintaining animals in their differential environments for a period of time twice the length of that typically employed in the behavioural literature and exposing them to the rigours of pregnancy and pup care, needed to be investigated to ensure that behavioural differences noted in the literature are maintained following these procedures. This was fulfilled in experiment two, in which post partum SEC animals were found to be behaviourally different from their SC and IC counterparts. Furthermore it appeared that in some instances (but not all) the effects of pregnancy had altered these differences. These results were discussed in the light of other findings in the post partum EC/IC literature and several future research recommendations were made.

CHAPTER SIX: STUDY TWO

THE EFFECTS OF DIFFERENTIAL MATERNAL ENVIRONMENTS PRIOR TO PREGNANCY ON OFFSPRING AND GRANDOFFSPRING BEHAVIOUR

6:1 GENERAL INTRODUCTION

The effects of environmental enrichment on both brain and behaviour have been the subject of systematic investigation since the late 1940's (Greenough 1976; Rosenzweig 1971; Rosenzweig and Bennett 1976; 1977; Renner and Rosenzweig 1987) and have been reviewed in chapters one and two of this thesis. To date, however, scant attention has been paid to the possible effects of enrichment on future generations. Indeed, within the large EC/IC literature only some three dozen studies [1] have considered this issue at all and for the most part, parental enrichment was an inadvertant methodological decision (animals being placed in differential environments whilst pregnant and often given direct exposure to enrichment post partum) rather than a direct focus of investigation.

In this chapter two experiments are described in which the effects of differential maternal environments prior to pregnancy were investigated over two generations. In particular, experiment one concentrated on the effects of enrichment of future *mothers* on their offspring's activity, learning and perceptual abilities, whilst experiment two examined the effects of enriching the *grandparent* generation on their grandpups.

6:2 EXPERIMENT ONE

6:2:1 INTRODUCTION

Within the EC/IC literature, of the few studies in which the effects of enrichment of the parent generation on their offspring was the subject of deliberate investigation, rather than an incidental finding, only three (Denenberg and Rosenberg 1967; Diamond 1984; Diamond, Chui, Johnson, Chelgren, Greer and Gibbons 1984) employed a procedure similar to that used in the present work, namely exposing females to differential environments *prior* to pregnancy. The importance of this type of procedure has been described in detail in chapter one, but is worth repeating here.

[1] See Chapter one for review.

Manipulating the mother's environment prior to pregnancy ensures that any alterations observed in the offspring must be mediated by the impact of this manipulation on the mother and not as a result of the manipulation impacting directly on the offspring. The remainder of the studies either employed prenatal and/or perinatal environmental experience, both of which methodologies could affect the offspring directly, rather than indirectly via the mother (these studies are tabulated in chapter one, but see chapter three for full discussion of prior to conception influences).

Of the three studies employing prior to pregnancy procedures, two, (Diamond 1984; Diamond et al 1984), were concerned with anatomical changes in offspring, only Denenberg and Rosenberg's work investigating the *behavioural* consequences of enriching the parent generation. As this latter work provides the only study in the literature which is directly comparable to the present research, it will be described in some detail[2].

Denenberg and Rosenberg (1967) employed a complex breeding design, in which half the grandmothers of the experimental subjects were handled, the remainder serving as non-handled controls. The mothers of the experimental subjects were either born into maternity cages or enriched environments and post weaning, maintained in EC's or standard laboratory cages until 50 days old. At 150 days of age, these future mothers were mated and their offspring born into maternity cages. At birth litters were reduced to eight pups and at 21 days placed into an open field for one three-minute trial and weighed. Table 6:1 summarises the experimental design, for each of the eight treatment combinations.

The data for activity and weaning weights revealed that handling females in infancy had a significant effect two generations on. Furthermore, the nature of the mother's living quarters during her early life profoundly affected her offspring and these variables acted in a non-additive interactive manner.

[2]This study has been mentioned in chapter one, but is worth a more extensive overview at this point in the thesis.

HANDLING EXPERIENCE OF GRANDMOTHERS OF EXPERIMENTAL SUBJECTS	PREWEANING HOUSING OF MOTHERS OF EXPERIMENTAL SUBJECTS	POSTWEANING HOUSING OF MOTHERS OF EXPERIMENTAL SUBJECTS
Non-Handled Non-Handled	Maternity Cage Maternity Cage	Laboratory Cage Free Environment
Non-Handled Non-Handled	Free Environment Free Environment	Laboratory Cage Free Environment
Handled Handled	Maternity Cage Maternity Cage	Laboratory Cage Free Environment
Handled Handled	Free Environment Free Environment	Laboratory Cage Free Environment

Table 6:1 Breeding Design employed by Denenberg and Rosenberg 1967

More specifically, however, the results were complex. With respect to the activity measure, descendants of non-handled grandmothers were more active than descendants of handled grandmothers if their mothers had been reared in a maternity cage between birth and weaning. Exactly the opposite pattern was obtained if their mothers had been reared in a free environment during infancy. The grandmother handling by mother's postweaning housing interaction was also significant, the pattern being the opposite to that just described above. In addition, the preweaning housing by postweaning housing interaction was significant. Offspring of mothers reared in two different environments (ie: cage and free environment, or free environment and cage) were more active than offspring of mothers reared only in cages, or reared only in free environments for the first 50 days of life.

With the other behavioural measure, weaning weight, the two main effects of grandmother handling and maternal postweaning housing were also significant, as was their interaction. Weanlings whose grandmothers were not handled, and whose mothers were raised in laboratory cages after weaning [3], weighed significantly more than the other three groups.

One obvious problem with Denenberg and Rosenberg's work, which is of particular concern to the present research, is the difficulty there is in extracting information about the *postnatal* maternal experience on offspring. This is particularily true of the activity data, where postnatal effects

[3] This group is equivalent to the present thesis' SC offspring.

emerge only as part of a complex interaction. The value of this work to the present thesis, however, lies quite simply in the fact that it was the first study to report significant effects of differential maternal environments prior to pregnancy on offspring behaviour, the details of which remain to be elucidated. Consequently, Denenberg and Rosenberg's work can be considered as one of the starting points of this thesis, which aims to investigate the *nature* of the offspring behaviour.

In this chapter, therefore, it was decided extend this early work, by investigating offspring weaning weights and open field behaviour, as well as their Skinner box and visual cliff performance. These latter two tests were chosen, as they have been found to reliably differentiate between animals exposed directly to superenriched, standard and and impoverished conditions in the previous chapter of this thesis, as well as in the EC/IC literature more generally (see chapter two).

6:2:2 METHODOLOGY

a) Subjects:

These were 60 male and 60 female F2 generation Hooded Lister rats of weanling age (19-21 days), 20 of each sex being bred from F1 generation females exposed to SEC, SC or IC environments prior to pregnancy. Details of the breeding procedures and environments employed can be found in the general methodology chapter (Chapter 4). Because of the large number of animals to be used in this experiment, it was decided to breed them in two batches, care being taken to replicate all procedures. Equal numbers of males and females from each of the three experimental groups were obtained from each batch to ensure counterbalancing, and thus equate the groups in terms of any possible interfering factors, for instance disturbance in the colony room, or effects due to seasonal variations. In this and all subsequent experiments care was taken to ensure that equal numbers of litters were used in each of the offspring groups.

b) Environments and Apparatus:

The environments employed in this experiment, as well as the open field, the visual cliff and the Type II Skinner box system are all detailed in the general methodology chapter, and are identical to those used in chapter 5.

c) Procedure:

At weaning, subjects were weighed and assigned to individual cages for a day prior to the start of testing. This was to allow them to settle following separation from their mothers and siblings and also to allow a technician to recode them, so as to disguise their experimental backgrounds from the experimenter. Starting at 22 days of age, all 120 subjects (run in two batches of 60) were given five daily three-minute trials in the open field, following the procedure described in chapter six. Number of lines crossed, rears, defecations and time spent in the centre of the open field were recorded, running order for the animals being randomised at the start of each day of testing. During this time animals were maintained on an ad libitum diet.

At the end of the open field testing, subjects were divided into two separate groups such that equal numbers of animals from each litter, sex and experimental background were represented in each group. These two groups then underwent different procedures, the first group being assigned to the visual cliff task and the second to the Skinner box apparatus. On day 26, the visual cliff group were given two trials, one with the cliff side of the apparatus set at one inch below the glass, the other with the cliff set at 12 inches below the glass. Order of trial presentation was randomised for each animal and the procedure for each trial, detailed in chapter six, employed. Two measures were recorded, latency to descend onto the cliff from a central barrier and side chosen. On the same day, those subjects allocated to the Skinner box task were deprived of all food for three hours in the afternoon and given eight grams of breeding diet in the evening.

On day 27, these latter animals started a six day procedure of Skinner box testing, being given

eight grams of food at the end of each day of testing. As the animals were very small, they were weighed twice a day to ensure that they were following a normal growth pattern and that the 12 hour deprivation diet was not adversely affecting their health. Subjects received one 30 minute Skinner box trial on each of the six consecutive days, reinforcement consisting of one pellet of food paired with one second illumination of the four house lights. The following training schedule was employed: DAYS 1 and 2-CRF; DAYS 3 and 4-FR3; DAY 5-FR6; DAY 6-FR9. Subject order within each day was randomised, and number of bar presses recorded.

6:2:3 RESULTS

In contrast with many studies of the effects of early experience on later behaviour and as described in the general methodology chapter of this thesis (chapter four), the practice of culling to equate litter sizes was not employed in the present work. Following Fride et al's (1986) recommendation, in order to prevent littersize effects, no more than two male and/or female littermates were used for a particular test. Furthermore, to ensure that any littersize variations existing between experimental conditions were not significant, a statistical procedure described in more detail in the general methodology chapter was employed. To ascertain littersize effects a two factor analysis of variance on size of litter from which each subject was drawn was carried out prior to analysis of the individual behavioural measures. This failed to reveal any differences either between environmental condition $F(2,114)=0.70$ $p>0.05$ or between males and females $F(1,114)=0.00$ $p>0.05$, establishing that any effects contributed by littersize were equally distributed between the six offspring groups. Consequently, as recommended by Denenberg (1977) individual subjects were used as the unit of analysis in this experiment and no additional statistical checks were imposed on the data. For a further discussion of the rationale underlying these decisions, the reader is referred to chapter four.

Overall, the present data clearly demonstrate that manipulation of the home environment of female rats prior to pregnancy can significantly influence the behaviour of their offspring. In

detail, however, results are complex and will be dealt with more specifically under the individual tests employed.

a) Weaning Weights:

A two factor analysis of the weaning weights of the 120 animals employed in this study revealed no significant differences either between the three environmental groups $F(2,114)=0.95$ $p>0.05$, or between the sexes $F(1,114)=0.69$ $p>0.05$. As can be seen fom Table 6:2, which details the means of the six experimental groups, weanling rats' average weights are clustered around the 40gm mark.

SEC Males	42.45	SEC Females	41.85
SC Males	43.55	SC Females	41.80
IC Males	40.90	IC Females	38.85

Table 6:2 Mean weaning weights of the six offspring groups.

b) Open Field:

Analysis of variance of the lines crossed measure over the five days of open field testing revealed no significant main effects between subjects, no differences emerging between the environmental groups $F(2,114)=1.23$ $p>0.05$, or between male and female offspring $F(1,114)=0.15$ $p>0.05$. However, there was a significant within subjects main effect due to days $F(4,456)=3.26$ $p<0.01$. Typically, in the literature however, it is the *patterns* of activity over days and the activity on day one which are considered important (Lamden 1985) and indeed in this experiment, this is where the significant effects emerged. As can be seen from Figure 6:1, which describes the patterns of activity of the three experimental groups over the five days of testing, offspring of standard housed mothers reduced their activity over days, whereas, both SEC and IC offspring did not

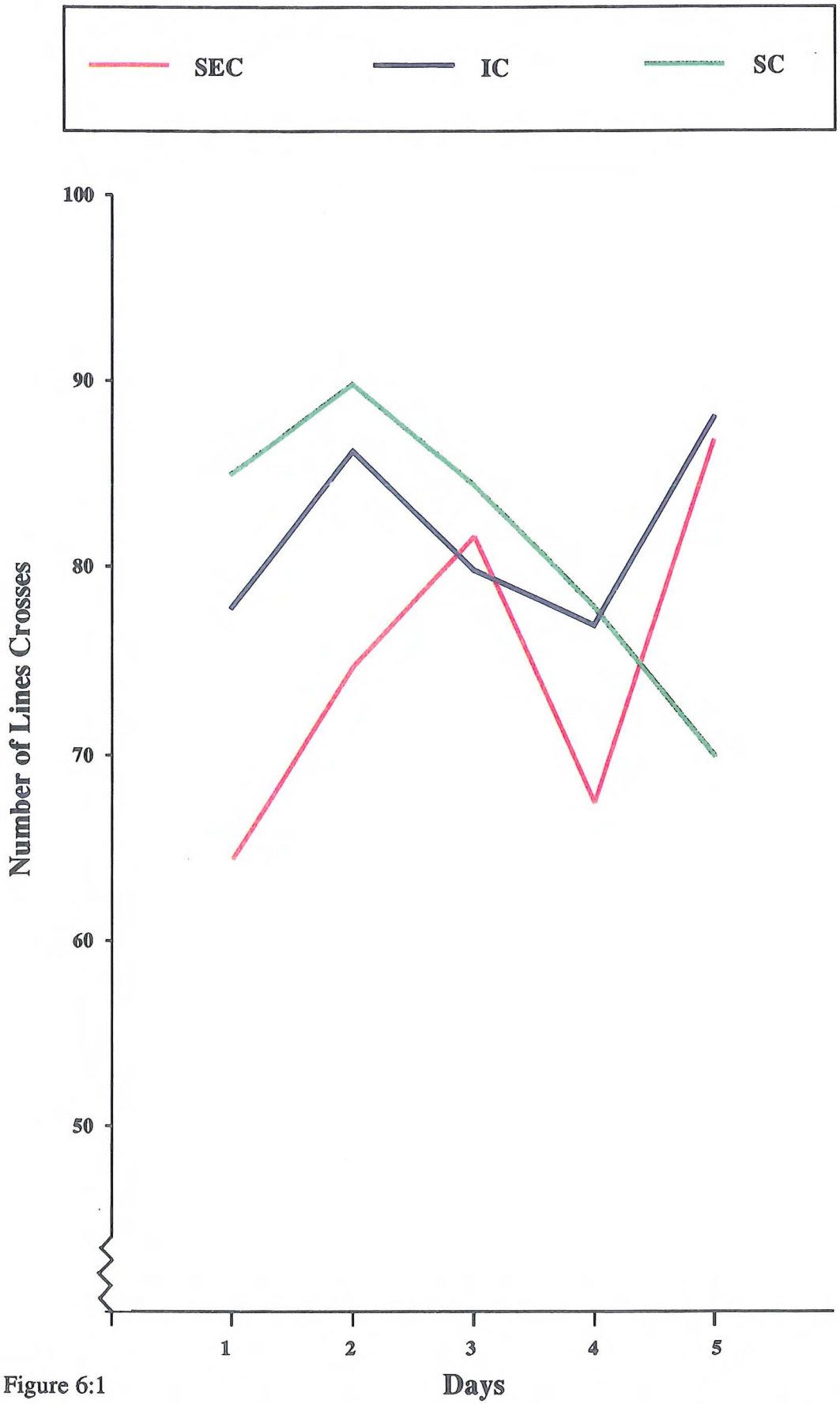


Figure 6:1

Mean number of lines crossed by the offspring of dams exposed to SEC, SC and IC prior to pregnancy, over the five days of open field testing. Male and female offspring groups' scores combined.
Key: SEC=offspring of SEC dams, SC=offspring of SC dams, IC=offspring of IC dams, for this Figure and Figures 6:2, 6:3 and 6:4.

appear to habituate to the apparatus, producing higher levels of responding on day five than day one. These differential patterns were highly significant, as revealed in the days by maternal environment interaction $F(8,456)=3.63$ $p<0.001$ and warranted further investigation.

In particular, as day one activity has been found to be related to emotionality (Whimbey and Denenberg 1967b) and as the offspring groups' performance appeared to differ when Figure 6:1 was consulted, post hoc analyses of the day one [4] data for the three offspring groups was carried out using the Newman Keuls test. Offspring of both SC and IC dams were significantly more active than their SEC counterparts ($p< 0.01$ and $p<0.05$ respectively) but did not differ significantly from each other.

With regard to the second open field measure to be analysed, number of rears, all three main effects, sex, maternal environment and days were significant $F(1,114)=4.17$ $p<0.04$; $F(2,114)=8.30$ $p<0.001$; and $F(4,456)=9.92$ $p<0.001$ respectively. The groups differences were further analysed post hoc using the Newman Keuls test and IC animals were found to rear significantly more than both SEC offspring ($p<0.01$) and SC offspring ($p<0.01$), the latter groups not differing significantly from each other. Females reared more than males.

As with the lines crossed measure, however, significant differences in patterns of responding over days also emerged, as revealed by the days by maternal environment interaction $F(8,456)=3.83$ $p<0.001$. Figure 6:2 which describes this interaction clearly shows that both IC and SEC descendants increased their rearing behaviour over days, whereas SC offspring maintained a fairly constant level of rears. None of the other interactions was significant. For full details of this and other ANOVAs the reader is referred to the appendix.

[4] Although day five also warrants post hoc investigation there are no theoretical precedents in the literature to justify this analysis, so none was done.

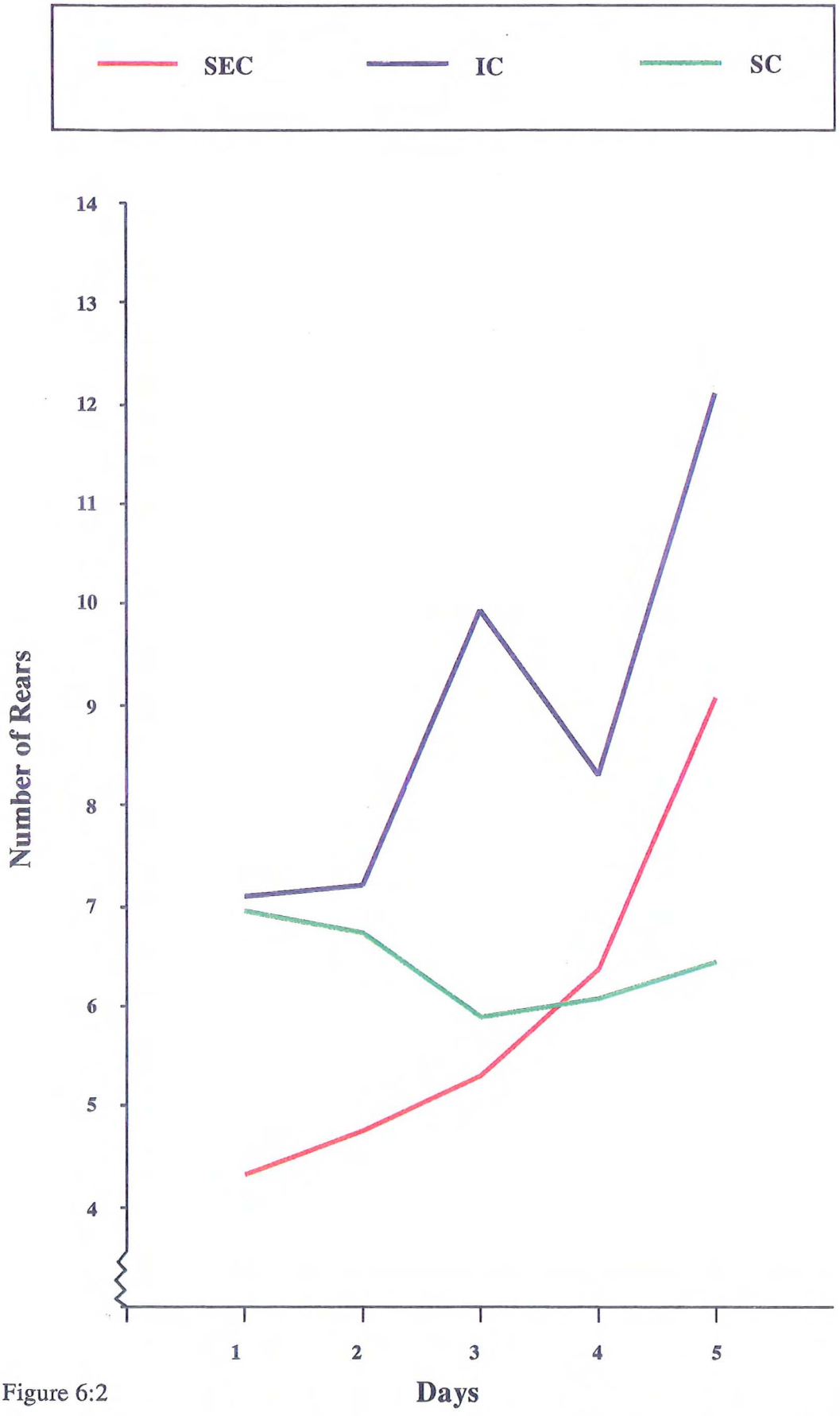


Figure 6:2

Mean number of rears by the three offspring groups over the five days of open field testing, male and female groups' scores combined.

Time spent in the centre of the open field, often considered as a measure of emotionality (Archer 1973) was also analysed and revealed significant differences between the experimental groups $F(2,114)=3.66$ $p<0.02$. Post hoc analysis revealed that IC descendants spent considerably less time in the centre circle than either their SEC ($p<0.05$) or SC ($p<0.05$) counterparts, the latter two groups not differing significantly from each other. In addition there was also a significant days effect $F(4,456)=6.89$ $p<0.001$, whilst as is increasingly common, the patterns of behaviour over days for the three groups were also significantly different from each other $F(8,456)=2.57$ $p<0.01$. Interestingly, in this measure no sex differences emerged $F(1,114)=0.06$ $p>0.05$. However, in the final open field measure to be considered, number of defecations, clear sex differences did emerge $F(1,114)=3.66$ $p<0.05$, males defecating more than females. In this last measure, no other significant differences emerged either between experimental groups $F(2,114)=0.23$ $p>0.05$ or over days $F(4,456)=2.6$ $p>0.05$.

c) Visual Cliff:

Table 6:3 details the number of offspring from the three experimental groups choosing deep and shallow sides in the two visual cliff trials, when the moveable shelf was set at either 12 inches or 1 inch below the glass.

	SEC(1)	IC(1)	SC(1)	SEC(12)	IC(12)	SC(12)
SHALLOW	13 (65)	9 (45)	12 (60)	13 (65)	6 (30)	11 (55)
DEEP	7 (35)	11 (55)	8 (40)	7 (35)	14 (70)	9 (45)

Table 6:3 Number of offspring (percentages in brackets) from the three experimental groups choosing either the deep or shallow side of the visual cliff when the deep side of the cliff was set at either 1 inch or 12 inches

Although there was a tendency for the offspring of the enriched animals to chose the shallow side, irrespective of whether the deep side of the visual cliff was set at either 1 inch or 12 inches, a Chi squared analysis for k independent samples on each of the two trials failed to find any significant differences between the three experimental groups. Maternal experience prior to pregnancy does

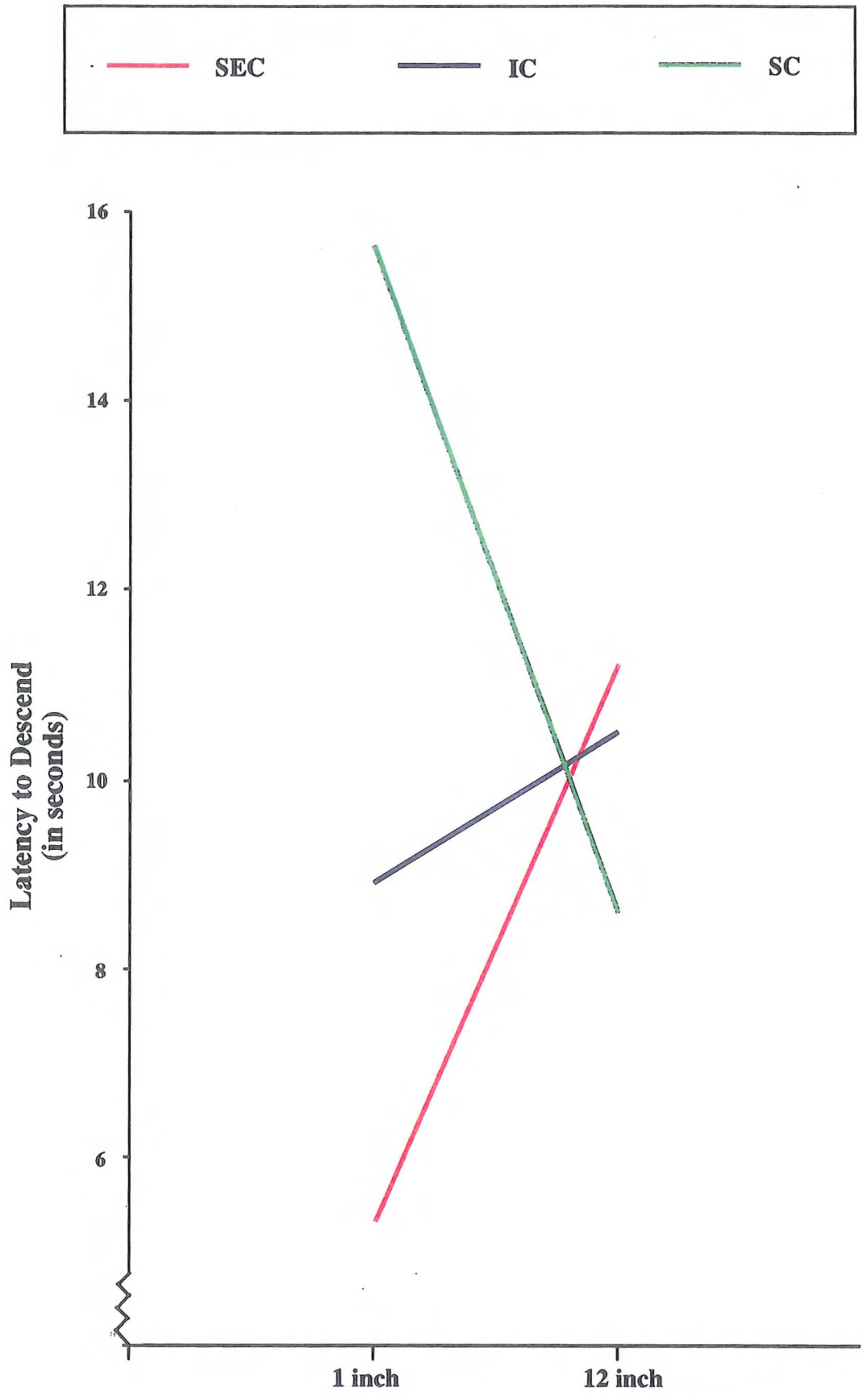


Figure 6:3

Time taken (in seconds) by the three offspring groups to descend onto the visual cliff when the "deep" side was set at both one and twelve inches, male and female groups' scores combined.

not appear, therefore, to affect offspring's visual cliff performance with respect to the side chosen measure.

Turning to the second visual cliff measure, latency to descend onto the cliff, ANOVA of the times taken to descend over the two trials, for the three environmental groups, although revealing no significant main effects due to the animals' background $F(2,54)=0.86$ $p>0.05$, did reveal a trials by background interaction $F(2,57)=4.41$ $p<0.02$. As can be seen from Figure 6:3, which presents this interaction graphically, both the SEC and IC groups took longer to descend when the visual cliff was set at 12 inches compared with the baseline 1 inch depth, the opposite being true for the SC group. That is, relative differences in performance over trials between the three groups reflected their parental backgrounds. In this study no sex differences emerged with respect to latency to descend $F(1,54)=1.78$ $p>0.05$, nor were any of the other interactions significant.

d) Skinner Box:

As can be seen from Figure 6:4, which shows the learning curves of the six offspring groups over the six days of testing, differential maternal environments produce offspring with considerably different patterns of responding. Analysis of variance of the bar press data revealed significant between subject main effects due to environmental background $F(2,54)=3.53$ $p<0.03$; sex $F(1,54)=5.33$ $p<0.02$ and an expected learning effect over days $F(5,270)=37.71$ $p<0.001$. In addition two significant interactions emerged, days by sex $F(5,270)=5.64$ $p<0.001$ and days by maternal environment $F(10,270)=2.95$ $p<0.001$. Taking the days by sex interaction first, from Figure 6:4, it can be seen that on the whole, male offspring have steeper learning curves than their female counterparts. This is consistent across all three offspring groups as evidenced by the lack of a groups by days by sex interaction $F(10,270)=0.41$ $p>0.05$. Considering the second significant interaction, groups by days, it is obvious from the graph that the three experimental groups have different learning rates. In particular, SC offspring have a faster acquisition of higher ratio schedules than do either IC or SEC offspring.

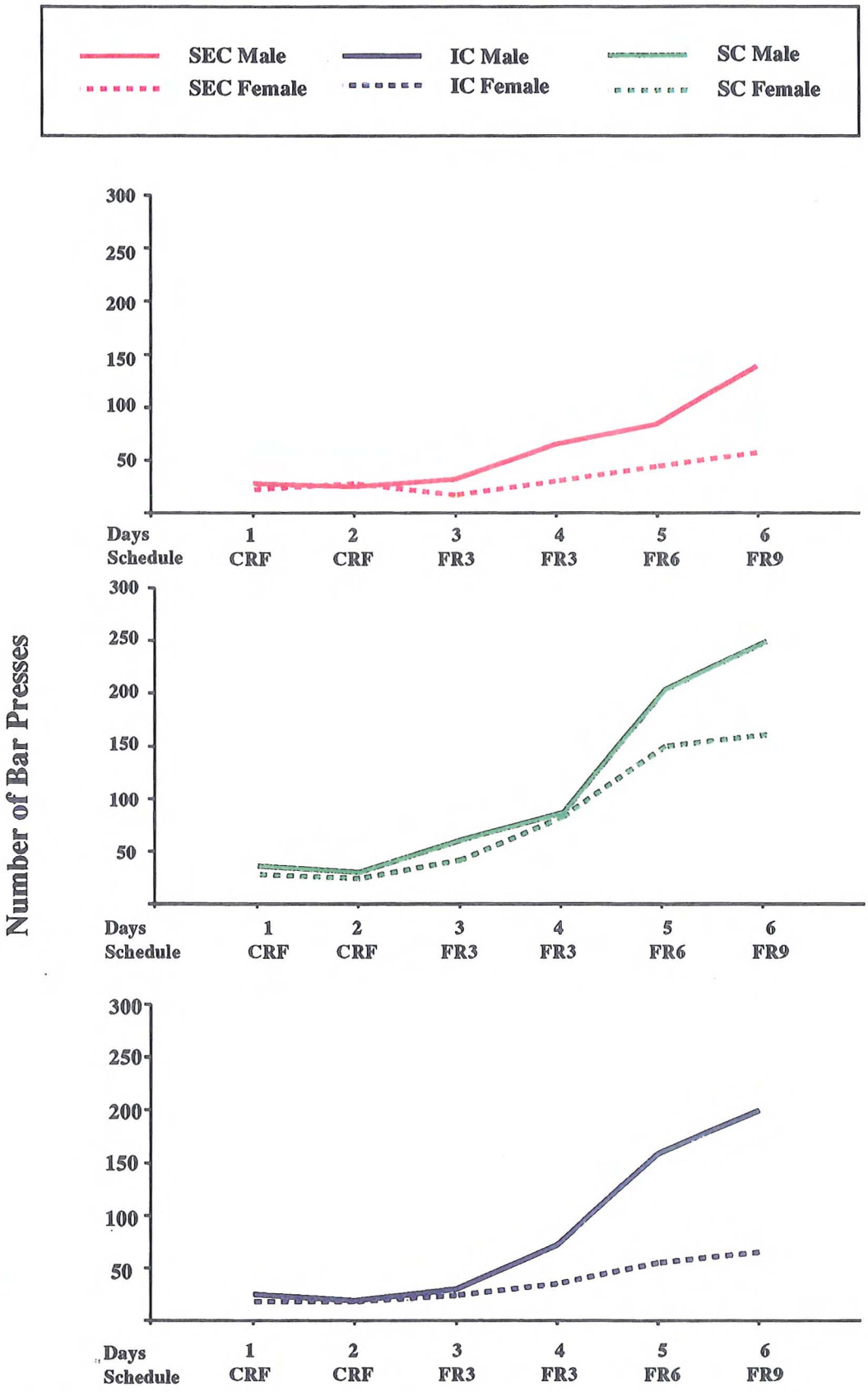


Figure 6:4

Mean number of bar presses by the six offspring groups over the six days of Skinner box training. Data presented according to gender and maternal environment.

Post hoc Newman Keuls tests on the significant group main effect confirms this, with SC animals differing significantly from SEC offspring ($p<0.05$), but not from IC offspring.

e) Summary of Significant Differences:

Table 6:4 describes the significant differences that have emerged in this experiment. Considering first the open field apparatus, on day one both SC and IC offspring crossed significantly more lines than their SEC counterparts, but did not differ statistically from each other. Offspring groups' patterns of responding over days also varied, SC animals habituating to the open field and reducing their number of lines crossed, when compared with their IC and SEC counterparts. However, IC offspring reared more than either the SEC or SC groups, the latter two groups not being statistically different from each other. With respect to the amount of time spent in the open field, IC descendants spent less time in the centre of the field than either of the other two groups.

APPARATUS	MEASURE	ANALYSIS	COMPARISON	PROBABILITY
Open Field	Lines Crossed	Newman Keuls (Day 1)	SEC vs IC SEC vs SC	$p<0.05$ $p<0.01$
Open Field	Lines Crossed	ANOVA-5 Days	Group by Days	$p<0.001$
Open Field	Rears	Newman Keuls	SEC vs IC SC vs IC	$p<0.01$ $p<0.01$
Open Field	Time in Centre	Newman Keuls	SEC vs IC IC vs SC	$p<0.05$ $p<0.05$
Visual Cliff	Latency	ANOVA	Group by Trials	$p<0.02$
Skinner Box	BarPress	Newman Keuls	SEC vs SC	$p<0.05$

Table 6:4 Summary of significant main effects of experiment one.

Moving on to the visual cliff, SC offspring took longer to descend when the visual cliff was set at 1 inch compared with 12 inches, the opposite pattern emerging in the SEC and IC offspring. No other significant differences emerged. With the Skinner box procedure, SC animals consistently bar pressed more than the SEC animals, the IC animals' bar press rates falling between these two groups but not differing significantly from either of them.

6:2:4 DISCUSSION

The present data clearly demonstrate that manipulation of the degree of enrichment of the environments of female rats prior to mating can significantly influence the behaviour of their offspring. In detail, however, the results are complex and difficult to interpret. Whilst some differences in offspring behaviour due to variation in maternal environment emerged as a main effect in four of the experimental measures (lines crossed, day one; rears; time in centre; bar presses) and as part of an interaction in two of the remaining five dependant variables (lines crossed over five days; latency to descend in the visual cliff apparatus), there was a lack of consistency in the pattern of differences between the three groups. In order to clarify offspring performance, results will be discussed under discrete headings with reference to both the EC/IC literature and the prenatal stress literature, where appropriate.

Offspring Weights:

In this experiment, the only physiological measure taken was that of offspring weaning weight. Unlike Denenberg and Rosenberg's (1967) results in which offspring of mothers raised in group cages (SC) were heavier than offspring of mothers exposed to free environments, in the present work no significant differences emerged between the three experimental groups. Furthermore, there were no differences between male and female offspring. This apparent discrepancy in results between the two studies may reflect procedural differences. For example, the present work did not cull litters, whereas the methodology employed by Denenberg and Rosenberg (1967) did. It may be therefore, that with smaller litters (resulting from culling) size differences due to maternal environment are able to emerge in the offspring, but in unculled litters, which by their very nature may be larger, these types of effects are obscured. Size of litter, after all is one contributing factor to the successful growth of offspring. Furthermore, there is inconsistency in this literature, exemplified by the only other report of the effects of enriching the parent generation prior to pregnancy on offspring body weights which found that pups from *enriched*

parents had increased body weights at birth (Diamond 1984). As few procedural details were included in this latter report, it is difficult to compare it with the present work. However, it should be noted that one obvious difference is the stage of development at which the animals were weighed (birth weights versus weaning weights). Furthermore, in Diamond's experiment animals were returned to their differential environments once pregnant.

Manipulation of the maternal generation by exposing animals to enriched, standard and impoverished environments has not been confined to the period prior to conception. As noted in chapter one, there are several studies in which animals have been exposed to differential environments prenatally. Of those reporting weight measurements, the tendency has been for the offspring of EC animals to be heavier than their IC counterparts at birth (Diamond et al 1984) and their SC counterparts postweaning (Kiyono et al 1985), no differences emerging between SC and IC offspring in the latter study [5]. As yet there is no explanation for the discrepancy in findings between these studies, the findings of Denenberg and Rosenberg (1967) and the present research. It may well be, however, that the *timing* of the manipulation plays an important role in the mediation of intergenerational effects.

Within the EC/IC literature where animals are exposed *directly* to differential environments, however, results appear to be more consistent. Typically *isolates* have been found to weigh more than either their EC (Rosenzweig, Bennett and Diamond 1972a; 1972b; Lamden 1985) or SC (Morgan and Einon 1975; Einon, Morgan and Kibbler 1978) littermates, with this effect being present even in animals exposed to differential environments prior to weaning (Malkasian and Diamond 1971). Several reasons for these weight changes have been postulated (see chapter two). It therefore appears that the metabolic differences typically observed between EC, SC and IC animals as reflected in the body weight measure do not appear to pass on to their offspring. One implication of this finding is that the behavioural differences evident in the offspring in this study cannot therefore be seen as a consequence of some (trivial) metabolic factor, but must be caused by some other as yet undetermined mechanism.

[5] No further details were available in Kiyono et al's (1985) study.

Offspring Open Field Performance:

Unlike Denenberg and Rosenberg's (1967) study in which the effects of environmentally enriching the mother prior to pregnancy on their offspring activity levels were obscured by the effects of handling the grandmother generation, in the present work the methodology employed allowed quite specific comparisons between activity levels of the offspring groups to be made. Considering SEC and IC offspring groups first, both male and female IC subjects were more active than their SEC counterparts on day one of open field testing. In addition, over all five days of testing they consistently reared more than the SEC offspring and spent less time in the centre of the open field. This suggests that the IC offspring are both more active (or exploratory) and more emotional than their SEC counterparts, a finding which has been noted in animals exposed directly to EC and IC (Lamden 1985; Dell and Rose 1987) and appears to be in direct contrast to the behaviour observed in the parent generation in the previous study of this thesis (Figure 5:1). Animals exposed directly to the SEC were more active than their IC counterparts on day one of open field testing, as well as rearing more over days (Figure 5:3) [6]..

In this study, however, both SEC and IC offspring groups' *patterns* of responding in the lines crossed and rearing measures tended to increase over the five days of testing. This is in direct contrast to work with animals exposed directly to EC/IC (Joseph and Gallagher 1980; Rose, Dell and Love 1985; Lamden 1985) in which typically, over trials, IC animals maintain high levels of activity, whilst their EC littermates' levels of responding drops, as well as contrasting with the patterns of behaviour established in the parent generation of SEC and IC animals noted in the previous chapter.

When the SC offspring performance is included, the picture becomes more complex, as their behavioural profiles either contrast with both the SEC and IC progeny, or mimic that of the SEC group, depending on the the measure. In particular, with respect to the lines crossed measure,

[6] It should be noted, however, that in the previous study, patterns over days demonstrated that overall IC animals crossed more lines and maintained higher patterns of responding than their SEC counterparts in both the virgin and postpartum groups (Figures 5:2 and 5:6), suggesting that there are some similarities between animals exposed directly to impoverished environments and their offspring, when patterns over time are considered

SC offspring have significantly higher day one scores than the SEC progeny. However, unlike both the SEC and IC offspring groups, over the five days of testing their response rates dropped, reminiscent of the behaviour patterns of animals exposed directly to SEC (chapter five). These patterns differ from those observed in animals exposed directly to the differential environments (Figure 5:1), where SC animals have lower day one scores than the SEC animals (but similar scores to the IC animals), although they too reduced their number of lines crossed over days.

In the rearing and time in centre measures, however, SC offspring behave in a manner which is statistically indistinct from the SEC offspring, that is rearing less than IC progeny and spending more time in the centre of the open field. In the parent generation (especially in the female animals) the opposite is true, SC animals like the IC animals, producing significantly less rears than their SEC counterparts prior to pregnancy (Figure 5:4) or significantly more than their SEC counterparts postpartum (Figure 5:7).

The fact that there is such a discrepancy between parent and offspring generations, however, is not unexpected, given the very different "life experiences" these two groups of animals have had. As can be seen from the procedures outlined in chapters four and five, having been born to and raised by standard housed mothers, once weaned the parent generation were housed in qualitatively different environments. As the SEC, SC and IC provide differential opportunities for both social and object interaction (that is, are socially and perceptually different) it is not surprising that the SEC, SC and IC animals should be qualitatively different from each other.

With the offspring generation, however, the only procedural difference between the three groups was the type of mother they had experienced. This can be seen as having two components, firstly differential uterine environments and secondly differential postnatal interactions with mothers with qualitatively (and even quantitatively) different behavioural profiles (Muir et al 1985). That such "minimal" (McKim and Thompson 1975) changes in early experience can have such a profound effect on subsequent offspring behaviour is of considerable theoretical importance and the nature of these changes as well as their causes need to be elucidated further. Within the

EC/IC literature, a variety of possible causes for the effects of environmental enrichment and impoverishment have been postulated (Renner and Rosenzweig 1987) which might well be relevant to an understanding of the effects of differential maternal environments on offspring. Similarly, the prenatal stress literature, reviewed in chapter three of this thesis, offers some understanding of intergenerational influences. Before investigating these possible causes further, however, the results of the offspring's performance in both the Skinner box and visual cliff apparatus require some discussion.

Offspring Visual Cliff Performance:

In the present visual cliff experiment, no statistically significant differences emerged between the offspring groups on the side chosen measure, results which are not entirely unexpected. Although significant differences have been reported in animals exposed directly to EC/IC (Lamden 1985; Eichengreen et al 1966), with EC animals appearing to have a more highly developed depth perception, this has been attributed to the fact that EC animals typically have more experience of depth from interaction with their environment, than their IC littermates. All the offspring groups employed in the present study had little or no experience of depth in their early environments and in any case had equivalent direct experience of depth. Consequently a lack of differences in depth perception is not unsurprising.

When comparing the side chosen measure in the offspring group with the results of the same measure in the SEC, SC and IC animals reported in the previous chapter (experiment one), however, one interesting contrast has emerged. Although neither the parent nor the offspring groups differed within their respective analyses, comparison of the two generations of animals' performances suggest that although animals directly exposed to IC were more likely to to chose the shallow side when the deep side was set at 12 inches, the opposite was true of the progeny of IC dams. Furthermore, of the six possible combinations of trials by environmental condition, this was the only reversal of the side chosen measure noted between parent and offspring generations.

It should be emphasised, however, that this offspring reversal was not statistically significant.

Moving on to the latency to descend measure in the offspring generation, although there were no overall differences between the groups, there was a significant trials by group interaction which warrants further discussion. As can be seen from Figure 6:3, when the cliff was set at 1 inch, offspring of SEC animals were quick to descend from the central barrier, IC progeny taking more time, the slowest animals being the offspring of SC dams. When the cliff was set at 12 inches, however, a very different pattern emerged. In this instance, both SEC and IC offspring tended to be both more cautious than the SC offspring and took longer to decide which side to go onto than when the cliff was set at one inch.

When the offspring latency performances in the visual cliff are compared with those of their parent generation, again differences in patterns of responding are apparent. In both the 1 inch and 12 inch trials, animals directly exposed to SEC were quicker to descend from the central barrier than either their SC and IC counterparts. That is, the only similarity between the two generations' patterns of responding emerged in the 1 inch trial, their relative patterns of responding over the two trials being very different.

So one obvious question is what do these performance differences mean? Latency to descend, as a behavioural measure has been interpreted in various ways. Lamden (1985) for example, has suggested that it reflects animals' decision times, whereas Curry (1987) emphasised the similarities between latency to descend and emergence behaviour. Furthermore, Routtenberg and Glickman (1964) have noted that as well as depth perception, visual cliff performances are influenced by emotionality and exploratory tendencies. All of these elements could be contributing to the present results. For example, if latency to descend is simply a measure of decision time, then as IC and SEC offspring took less time to decide when the cliff was set at one versus twelve inches, when compared with their SC counterparts, it would be reasonable to assume that these animals were responding to the depth of the cliff, that is that they had better depth perception than the offspring of SC animals. However, the groups' performances in terms of the side chosen

measure does not support this idea. If SEC and IC animals did have better depth perception then they would be more likely to chose the shallow side than the SC offspring. This was not the case. With respect to the idea that longer latencies reflect either greater emotionality, timidity or exploratory behaviours, when considering the relative performances of the three groups when the cliff was set at one inch, behavioural patterns would suggest that SC progeny were more timid, emotional or exploratory than their SEC and IC counterparts. If this were true, these animals should also have had longer latencies when the cliff was set at twelve inches. They did not. At this stage in the discussion, therefore, it is difficult to interpret the visual cliff findings. Indeed, in view of the fact that no differences were found between the groups in terms of the frequency of side chosen measure and that any differences in latency only emerged in an interaction, it is probably best not to make too much of these findings until a clearer picture of offspring performances has been achieved.

Offspring Skinner Box Performance:

One of the most exciting findings in the early EC/IC work was that simply exposing animals to enriched environments improved their problem-solving performance in mazes (Hebb 1947; Forgays and Forgays 1952; Hymovitch 1952) and altered their Skinner box behaviour (Rose, Love and Dell 1986) [7]. One obvious question that this present thesis had to address was whether alteration of performance in learning situations in the maternal generation would influence offspring performance in learning tasks [8].

As can be seen from the Skinner box data, differences do emerge between the offspring groups, with respect to their performance in a learning task, but these differences were only significant between the SEC and SC offspring, and not between the SEC and IC offspring [9]. As can be seen

[7] Although it should be noted that it is still far from certain that differences in Skinner box performance represent altered learning ability. For a fuller exposition of this please refer to chapter two.

[8] If this was found to be the case, then it would have important educational and therapeutic implications, as well as providing a procedural tool to further investigate the causes of enrichment.

[9] Differences between the SEC and IC offspring would have been expected, after all, if there were to be a transfer of learning effects across generations.

from the graphical representation of these results (Figure 6:4), offspring of SC animals appeared to bar press more than the IC progeny (although this was not statistically significant) as well as the SEC offspring group. The fact that there was a tendency for offspring of SC animals to present different behavioural profiles than offspring of either SEC or IC dams is reminiscent of their lines crossed behaviour in the open field and their latency to descend in the visual cliff. In both of these measures too, there was some evidence of SC offspring performing differently from their SEC and IC conspecifics.

So, if the differences observed between the offspring groups do not parallel those typically found in animals directly exposed to SEC, SC and IC (where to remind the reader, IC animals have been found to bar press more than their SEC counterparts, SC performances being qualified by sex of animal) one obvious question is what do these performance differences mean? A superficial analysis would suggest that SC offspring simply had superior learning abilities when compared with their SEC counterparts. However, one point, which is worth mentioning here, is that the Skinner box as a behavioural test may well be measuring animals' motivation to bar press rather than learning ability per se (Rose et al 1986; 1987). Consequently, at this stage, only speculative explanations of the offspring Skinner box performance can be proffered and offspring learning ability must be explored more specifically before any conclusions about maternally mediated improvements in cognitive capacity can be drawn.

As an initial test, the Skinner box apparatus is valuable, in that it is easy to conduct and has an extensive literature against which results can be compared. However, in order to fully investigate the learning performance of SEC, SC and IC offspring, learning tasks which either investigate problem solving ability (such as the Hebb Williams maze), or which equate intergroup motivation (Rose et al 1986; 1987) would be more appropriate. This is taken into account in the next chapter.

Summary and Possible Causes:

In the present experiment, offspring of mothers exposed to differential environments prior to pregnancy were put through a battery of tests to investigate their performances in activity, perceptual and learning tasks. Results complimented the early work of Denenberg and Rosenberg (1967) in that there was evidence of differences between the offspring of mothers previously housed in SEC, SC and IC, in all three tests. In addition by manipulating the maternal generation prior to pregnancy rather than placing *pregnant* animals in differential environments (McKim and Thompson 1975) or manipulating the mothers whilst pregnant and then rearing the pups in the environments too (Whimbey and Denenberg 1966; 1967a; Ravizza and Herschberger 1966; Denenberg, Woodcock and Rosenberg 1968; Denenberg and Whimbey 1968; Manosevitch and Montemayor 1972; Manosevitch and Joel 1973; Manosevitch and Pryor 1975; Sjoden and Soderberg 1975; Ivinskis and Homewood 1980; Venables et al 1988) the impact of the maternal experience itself is highlighted without the confounding effect of direct influence on the offspring.

Throughout this discussion, patterns of differences between the offspring groups have been compared with the patterns of differences that have been found in the parent generation. Two striking things have emerged. Firstly, that the differences in offspring performances are very different from those observed in animals exposed directly to SEC, SC and IC. Secondly, the behavioural profiles of the three offspring groups are complex, difficult to interpret and may reflect a variety of possible underlying mechanisms. Indeed, consideration of those few studies in which differential environments have been employed as the maternal manipulation (chapter one) highlights the various factors that have been considered as possible causes of the offspring effects, including biochemical, endocrinological and arousal differences between the maternal groups (Ivinskis and Homewood 1980; Kiyono et al 1985; Diamond 1987). Furthermore, consideration of the EC/IC literature itself reveals a wide range of factors which have been forwarded to account for the effects of *direct* exposure to differential environments which may indirectly contribute to the observed differences in the *offspring* groups. These include various *intervening variables* which

alter the internal state of the animal and which are held to be responsible for observed brain and behavioural changes (eg: maturation, stress, endocrine or neurochemical effects, arousal, learning and formation of memory) and *concrete types of behaviour* that are suggested as being necessary for the development of the typical EC-IC differences (eg: play, locomotion, response to social stimulation and object interaction) [10]. Obviously these are not mutually exclusive categories as the more mechanistic intervening variables can be considered as offering a different level of analysis from the more overt concrete behaviours and indeed, as noted by Renner and Rosenzweig (1987) the latter group may well be mediated by the former.

Considering environment-based explanations first, as the present methodology did not employ enrichment of the offspring (and indeed housed all the offspring in similar environments from conception) and all handling was standardised, differential locomotion, handling, object-interaction or extra-cage stimulation could not account for the present findings. Indeed the only difference between the three offspring groups was the type of mother they experienced which suggests that the most likely cause(s) of the behavioural differences observed in the present study either fall into the category of "intervening variables" in which the differential experiences afforded the mothers in some way affected the internal state of the offspring and produced the differences in performances, or the more concrete types of behaviours such as play and social stimulation perhaps induced by differential mothering.

These hypotheses are consistent with the findings from the large prenatal literature (reviewed in chapter three) in which maternal influences found to modify offspring characterisitics have been attributed either to changes in the physiology of the mother manifesting themselves as changes in the internal states of the offspring induced during the foetal period or after birth via the milk supply, or by behavioural changes in the offspring caused by modified maternal interaction (Denenberg and Whimbey 1963).

Considering the hypothesis that differential maternal environments affect the offsprings' internal

[10] This distinction between intervening variables and concrete types of behaviour was first suggested by Renner and Rosenzweig (1987).

states first, of the possible intervening variables that might account for the effects, some are more likely than others. It has been suggested, for example, that the observed brain differences between EC and IC subjects are attributable to different rates of maturation (Cummins, Livesey, Evans and Walsh 1978) and in particular that the increase in EC brain weight is the result of accelerated maturation. The same may be true of the offspring of EC animals. Indeed, Diamond et al (1984) have found that offspring of enriched parents had thicker cortices than their IC counterparts. However, in the present study, two indices of development, body weight and perceptual ability failed to reveal any significant differences between the offspring groups. Although this is not conclusive proof that differential maturation is not involved in the offspring effects, it does highlight other more probable causes, including endocrine system alteration, neurochemical changes, stress, differential arousal or learning.

In the present study no neurochemical or endocrine assays were taken and so no comments can be made about differences in the animals with respect to these measures. It should be noted, however, that endocrine system alteration has often been cited as a possible mediating variable in maternal manipulations other than enrichment (see chapter three) and may well contribute in this case too. Indeed in one of the few studies in which enrichment was employed as the prenatal manipulation (Kiyono et al 1985) "maternal biochemical changes" were implicated in mediating the differences observed in offspring performances in the Hebb Williams maze. What have been measured in this study, however, are the behavioural differences between offspring groups and these do allow some speculations to be made about possible internal states operating in the offspring.

One of the obvious explanations of the different behavioural profiles of the three offspring groups is to suggest that their mothers have been differentially stressed by their exposure to SEC, SC and IC. In reviewing the causes of the EC/IC effects (in animals directly exposed to these environments) Renner and Rosenzweig (1987) report that "Isolation is widely seen as a stressor, producing evidence of deleterious effects such as caudal dermatitis, aggressiveness and enlarge-

ment of the adrenals" (p73). However, the hypothesis that stress is an intervening variable in EC versus IC brain and behaviour differences has failed to produce clear cut results. For example, although Krech et al (1966) have reported that animals housed singly in extreme isolation have greater adrenal weights than EC animals, Wallace et al (1986) have reported the opposite. Magnitudes of EC versus IC cerebral effects have not been found to be changed by chronic stress (Riege and Morimoto 1970) and the general consensus of opinion to date is that although adrenal influences (regarded as indicative of stress) can co-occur with environmental experiences, they do not play a role in mediating the EC-IC effects. It may well be, however, that the stressors associated with being housed in isolation, or in an enriched environment which is both challenging and provides "a degree of uncertainty for the animals who reside there" (Renner and Rosenzweig 1987 p73), although not directly causing the observed EC/IC differences in animals exposed directly to the experience, do have an impact on their offspring.

Indeed, comparison of the present results with the effects of other stressful manipulations [11] imposed prior to pregnancy does appear to offer some evidence that SEC, SC and IC offspring are reacting as if they are differentially stressed. For example, Denenberg et al (1962) have found that shocking future mothers in infancy results in significant emotionality in their offspring when compared with controls, reminiscent of the behaviour of the IC progeny. However, not all the evidence is supportive. Thompson et al (1962) reported no differences in ambulation between their stressed and control offspring, unlike the patterns observed between the present offspring groups. Obviously the nature of the stressor plays an important role in the transfer of effects across generations (see chapter three). The main problem in postulating that stress causes the offspring effects, however, is the lack of predictable pattern between the SEC, SC and IC offspring behaviour across tasks. If the offspring groups were differentially stressed then clearer behavioural profiles should have emerged both within and across tasks. This was not the case. Of course it may be that maternal stress is having an impact on the offspring, but from the data, it cannot be the sole cause of the differences.

[11] See for example Thompson et al 1962; Ader and Belfer 1962b; Joffe 1965b; Ressler 1966; Denenberg et al 1962; Gauron 1966; Pereira et al 1980; Lane and Hyde 1973; Denenberg and Whimbey 1963; Morse 1979.

The idea that offspring of differentially housed mothers are differently stressed, however, is not the only potential explanation for the present results. Certainly, within the literature it appears that differential environments, as well as modifying their incumbents' neuroanatomy and neurochemistry, coupled with activity and learning performances, can also affect their maternal behaviours. For example, it has been reported that animals allocated to enrichment *postpartum* with their litters, exhibit qualitatively different maternal behaviour than mothers exposed to a standard postpartum environment [12]. In particular, the enriched mothers spend less time in the nest and less time nursing than control mothers, but on return to the nest spend more time interacting with their pups (Muir, Pfister and Ivinskis 1985). Given that the mother is an important agent in supplying stimulation and maintaining the arousal of her offspring (Ivinskis and Homewood 1980) and that it is quite likely that the differentially reared mothers will behave differently with their pups [13], then it is not unreasonable to assume that the offspring groups will be different. Furthermore, following Walsh and Cummins' (1975) arousal hypothesis, early exposure to stimulation resulting in raised arousal levels, can act as a "vaccine" immunising animals to future stimuli by altering their arousal homeostasis. It may be therefore, that the three offspring groups' baseline arousal levels have been altered by the stimulation afforded them by their qualitatively different mothers. As yet the precise nature of this stimulation is not clearly defined, but it is likely that these pups will in turn develop different behavioural profiles, a fact noted in the present work.

As an explanatory variable, arousal, although often discussed in the literature (Walsh and Cummins 1975), is not without its problems (Neiss 1988). For example, there has been confusion about the relationship of arousal to performance. Early researchers suggested that this relationship could best be described in terms of an inverted-U (Yerkes and Dodson 1908), optimal arousal producing optimal performance and both higher and lower than optimal arousal being associated

[12] In the present experiment maternal behaviour during the preweaning phase was not examined, as it was decided to minimise any disturbances of the litters during this stage of development, to avoid directly stimulating the offspring. However, this observation of litters might well be an area worth exploring in the future (see chapter nine)

[13] With for example enriched mothers' style of interaction providing additional stimulation of the infant's internal arousal system and thus "enriching" their pups when compared with the behaviour of SC or IC mothers.

with lower levels of performance. Later, in a critical appraisal of this hypothesis, Landers (1980) emphasised that the inverted U was not an explanation for the arousal-performance relationship, merely a description of it.

This debate notwithstanding, an arousal hypothesis provides an obvious framework within which to consider the present findings. Certainly some aspects of the data could be seen in terms of differential arousal levels in the three offspring groups, interacting with an optimal arousal level for a particular task. However, the present data taken as a whole do not lend themselves readily to interpretation in terms of inverted-U functions, even allowing for different levels of arousal being optimal in different test situations and for the two sides of the U not necessarily being symmetrical. For example, the rank ordering of offspring groups showed little consistency even within a single test situation. Of course the operation of a differential arousal homeostasis in the three offspring groups could be but one of several factors determining performance in the test situations. Consequently it is difficult to reject an arousal theory, at least until further information is available.

One of the other factors which may be contributing to differences in offspring performance in this study is learning. Consistently found in the EC-IC literature is the idea that differential opportunity for learning is one of the major causes of the cerebral differences noted in animals exposed directly to environmental experience. Although Rosenzweig and Bennett (1976) state "it is of course not certain that these larger cerebral effects could be attributed to learning and memory as such" (p206), others such as Greenough (1976) maintain that learning must be involved in producing the EC-IC differences "it would be much more difficult to argue that EC rats do not learn more than their cagehoused counterparts than it is to argue that they do". There are several reasons that support the conclusion that differential memory formation is at least one component in producing these differences (comprehensively reviewed by Renner and Rosenzweig 1987) including the fact that both the behavioural and cerebral effects of environmental complexity appear to be potentiated by drugs that affect memory formation (Bennett et

al 1973). Indeed Gardner et al (1975) have outlined a number of types of learning that probably take place in an enriched environment including passive avoidance learning (avoiding aggressive behaviour of fellow animals), exploration of novel objects, spatial and depth discrimination, all of which might generalise to the open field and to learning tasks. It may well be therefore that the enriched mother, who is more adaptable to novel situations and can generalise her learning across experiences, produces a different influence [14] on her offspring than a non-enriched mother. Whether this easier adaption reduces maternal stress levels and resultant hormones, which in turn have an impact on the offspring (as would be suggested by the prenatal stress literature), or in some way produces maternal behaviours which afford the offspring more learning experiences, thus altering their abilities, are for the moment pure speculations. What is clear, however, is that there is a case for investigating further the performance of the offspring groups in a learning task.

Following on from the observation that enriched dams produce qualitatively and quantitatively different maternal behaviours (Muir et al 1985) from standard housed dams, is the idea that their offspring are subjected to maternal interactions which can be seen as different types of social stimulation and perhaps even opportunities for play. These types of social interaction have been implicated in the development of the typical EC-IC effects (Welch et al 1974; Einon et al 1978; 1981) and there is no reason to suppose that they would not have a similar effect on the offspring. However as noted before, in the procedure employed in this thesis, no recordings of mother-infant interactions were taken [15]. Consequently in the remainder of this thesis, only the more mechanistic hypotheses that purport to alter the internal state of the organism and which are testable in the offspring groups using behavioural measures (such as stress, arousal and learning) will be explored further.

Prior to investigating the offspring groups' behaviour in more detail, however, the effects of differential maternal environments across two generations, the second aim of this chapter, will

[14] For example the SEC dam may well interact with her pups in such a way as to maximise their learning opportunities

[15] This was a deliberate decision to minimise any disturbance of the mother during the postpartum period.

be described.

6:3 EXPERIMENT TWO

6:3:1 INTRODUCTION

In the preceding experiment, behavioural differences were found in offspring of animals exposed to SEC, IC or SC prior to pregnancy. One obvious question which arises from these results is whether or not these effects carry across more than one generation. In this present experiment, enrichment of the *grandparent* generation was investigated, a methodology that has few precedents in the EC/IC literature. Indeed, of the few studies that have reported the effects of enrichment across generations, only two have mentioned grandparent effects (Diamond 1984; Diamond, Chui, Johnson, Chelgren, Greer and Gibbons 1984). However, unlike the present work which concentrates on *behavioural* effects, both of these studies investigated *anatomical* changes in offspring. In particular, these authors reported increments in cortical thickness occuring in F1, F2 and F3 generations of pups whose parents were environmentally enriched. Consequently, the present experiment can be seen as a new departure in the literature. Given its emphasis on the behavioural rather than anatomical effects of enrichment across generations it has the possibility of extending our knowledge of both enrichment and in conjuntion with Diamond's work, the relationship between brain and behaviour.

Manipulations of the grandparent generation which do result in behavioural changes in the grand-offspring, however, although extremely rare, are not unknown, For example, within the prenatal stress literature reviewed in some detail in chapter three, two studies have been published in which grandmother effects were found (Denenberg and Rosenberg 1967; Wehmer, Porter and Scales 1970). Although neither of these studies employed enrichment as a maternal manipulation, Denenberg and Rosenberg employing handling and Wehmer at al, avoidance conditioning, their relevance to the present research lies in the fact that they demonstrate that manipulation

of the grandmother can result in behavioural effects two generations later. Indeed, Denenberg and Rosenberg have coined the phrase "non-genetic transfer of information across generations" to describe their findings.

In the present experiment the behavioural effects of exposing the grandmother generation to SEC, SC and IC prior to pregnancy on the grandpup generation was investigated [16], using two test apparatuses which clearly differentiated the *offspring* of differentially housed mothers, namely the open field and the Skinner box. In this experiment the visual cliff was not employed as there was a limited number of subjects available. Furthermore to put them through three behavioural tests, which would have been unavoidable in view of the small number of subjects, may well have had an enriching experience in itself, thus confounding results (Rosenzweig and Bennett 1977 have noted that training alters brain anatomy). Consequently the choice of tests was made based on priorities. Of particular interest to the present thesis, were activity levels and learning ability. Therefore, test situations which measure these behavioural components were chosen. In addition, weaning weights of the three groups were also recorded. Furthermore, because of the complexity of the breeding procedure and number of animals involved, it was decided to restrict this experiment to male grandoffspring only, reflecting the fact that most of the EC/IC literature typically employs male rather than female animals (see chapter two of this thesis). At this time only preliminary analyses of the data from the previous experiments had been carried out to ascertain if differences did exist between offspring, but to reduce experimenter expectancy effects (Rosenthall 1966) only the most basic analyses had been performed and the fact that sex differences had emerged in the previous experiment (as a main effect, but not as an interaction with environmental group) had not been discovered when this decision was taken. In retrospect, it may have been preferable to employ female grandoffspring too.

[16] The grandpup generation is simply the offspring of the generation of animals employed in the previous experiment of this study.

6:3:2 METHODOLOGY

a) Subjects:

These were 30 male F3 generation Hooded Lister rats of weanling age (19-21 Days), 10 from each of the three groups of F2 generation females maintained in standard group housing (SC), whose dams' had been exposed to either SEC, SC or IC prior to pregnancy. The small number of animals employed in this experiment was due to practical limitations. To breed even these few animals took up quite large amounts of space and resources in the laboratory a consideration that had to be taken into account throughout this thesis. Details of this breeding procedure can be found in the general methodology chapter (Chapter four).

b) Apparatus and Environments:

The open field and the Type II Skinner box system employed in this experiment were the same as were used in the previous experiment, details of which can be found in the general methodology chapter, along with descriptions of the three environments to which the grandmothers of the present experimental subjects were exposed.

c) Procedure:

Subjects were weaned and assigned to individual cages for a day prior to the start of testing, during which time their experimental backgrounds were coded by a technician, so that the experimenter was unaware of their environmental history. Starting at 22 days of age, all 30 subjects were given five daily three-minute trials in the open field, following the procedure employed in experiment one of the previous chapter. Number of lines crossed, rears and defecations and seconds spent in the centre of the open field were recorded, running order for the animals being randomised at the start of each day of testing. During this time animals were weighed daily to

ensure they were healthy and developing normally and were maintained on an ad libitum diet.

At the end of the open field testing, all subjects were placed on a maintenance diet of eight grams of food at the end of each day and starting on Day 29, embarked on an eighteen day Skinner box test procedure developed by Rose, Love and Dell (1986). In particular, subjects received one 30 minute Skinner box trial daily, reinforcement consisting of one pellet of food (Noyes pellets) paired with one second illumination of the four house lights. The training schedule employed was the same as in previous experiments, namely: CRF (Days 1 and 2); FR3 (Days 3, 4, 5 and 8); FR6 (Days 9, 10, 11 and 12); FR12 (Days 15, 16, 17 and 18). On Days 6, 7, 13 and 14 subjects were maintained on an ad libitum diet to ensure that they were not adversely affected by their deprivation diet. Subject running order was randomised on each day of testing and number of bar presses recorded.

6:3:3 RESULTS

As in experiment one of this chapter, the practice of culling to equate littersizes typically reported in experiential research was not employed in this present work, although equal numbers of litters were represented in the three groups. Consequently, prior to analysis of any of the behavioural data a oneway ANOVA of size of litter that each subject came from was carried out and revealed no significant differences between the three experimental groups $F(2,27)=2.03$ $p>0.05$. Consequently no further statistical corrections were employed to control for variance due to littersize as the contribution of this factor to the three groups was deemed equal. As with the previous experiment individual subjects were used as the unit of analysis following Denenberg's recommendations (Denenberg 1977).

With respect to the behavioural data, the present results demonstrate that the effects of differential maternal environments prior to pregnancy can have a behavioural impact on their grandoffspring, although these differences are far less robust than in earlier generations. In this experiment significant differences emerged between the groups' rearing behaviour in the open

field test and there was a tendency for SEC and SC grandoffspring to bar press more than their IC counterparts and to cross more lines in the open field, although the last two results were not statistically significant. These results, in conjunction with the weaning weights will be described in more detail below.

a) Weaning Weights:

A oneway analysis of variance of the weaning weights of the three experimental groups revealed no significant differences between grandoffspring of SEC, SC and IC dams $F(2,27)=0.853$ $p>0.05$. As can be seen from Table 6:5 which described the groups' weaning weights, grandoffspring weights are around the 50 gm mark.

GROUP	WEIGHT
SEC	52.20
SC	48.10
IC	50.90

Table 6:5 Grandoffspring weaning weights.

b) Open Field:

As can be seen from Figure 6:5, which graphically represents the mean number of lines crossed by the three experimental groups, there was a tendency for SEC and SC grandoffspring to cross more lines than their IC counterparts. However, this was not statistically significant $F(2,27)=2.67$ $p>0.05$. Predictably, however, there was a significant days effect $F(4,108)=6.07$ $p<0.001$, day two producing the highest levels of responding. Finally, although Figure 6:5 suggests differential patterns of responding over the days for the three experimental groups, the relevant interaction was not statistically significant $F(8,108)=1.15$ $p>0.05$.

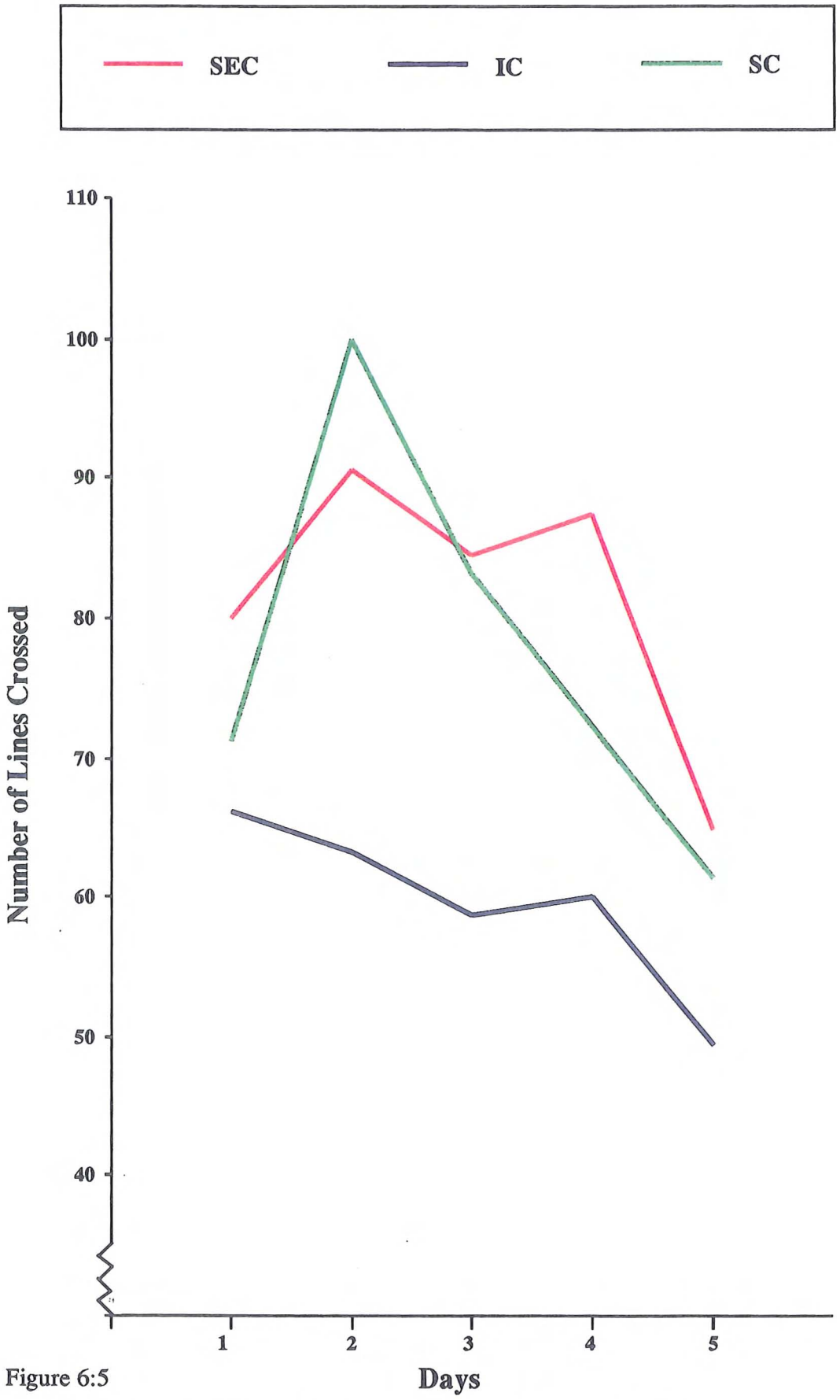


Figure 6:5

Mean number of lines crossed for the three grandoffspring groups over the five days of open field testing.
Key: SEC=grandoffspring of SEC dams, SC=grandoffspring of SC dams// IC=grand offspring of IC dams, for this Figure and Figures 6:6 and 6:7.

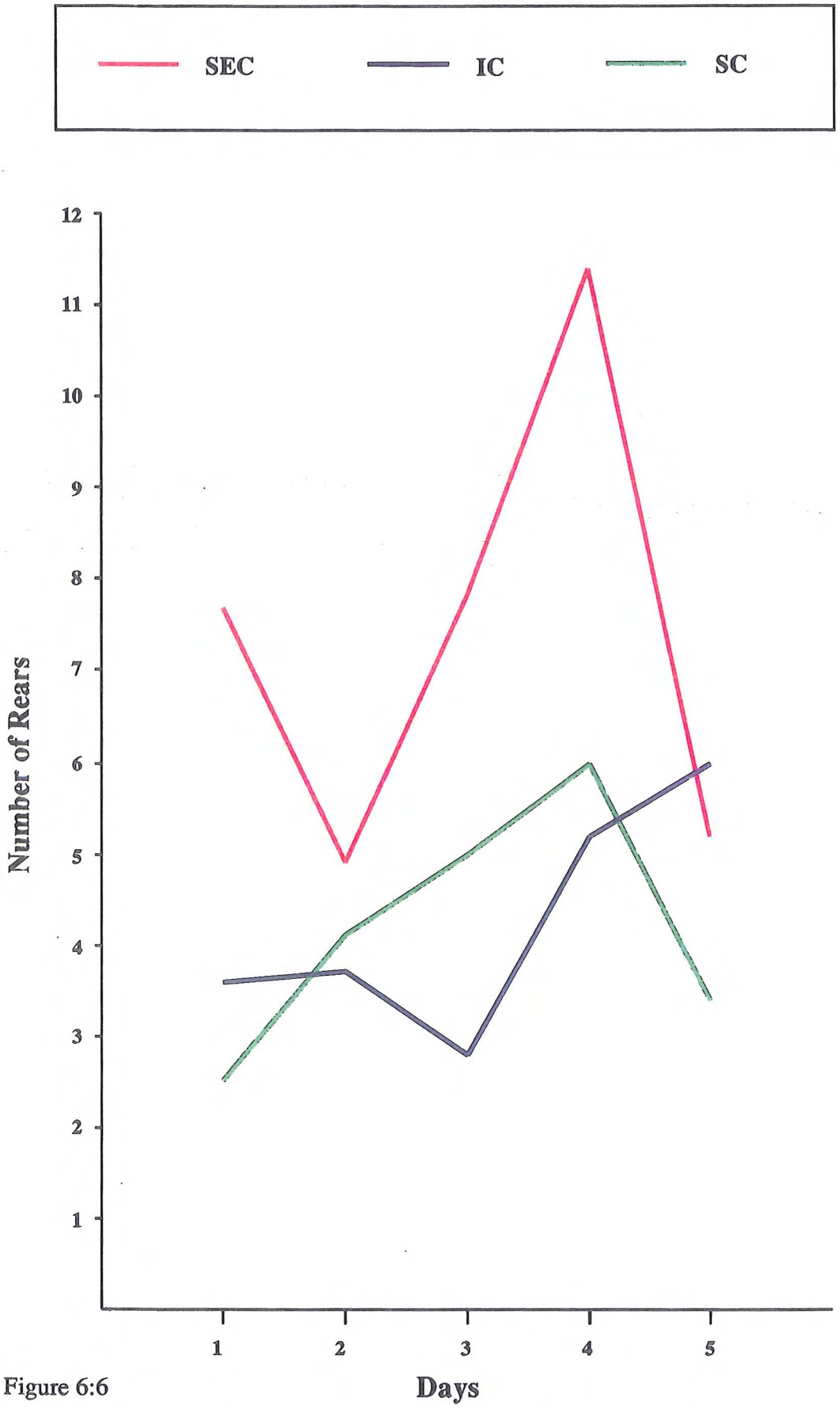


Figure 6:6

Mean number of rears by the three grandoffspring groups over the five days of open field testing.

With respect to the rearing measure, however, significant differences did emerge between the three experimental groups $F(2,27)=5.44$ $p>0.01$, qualified by a significant days by experimental group interaction $F(4,108)=2.95$ $p<0.005$. As can be seen from Figure 6:6, SEC grandpups reared significantly more than either their SC or IC counterparts, confirmed by a post hoc Newman Keuls analysis, $p<0.05$ and $p<0.01$, for the comparisons respectively. In addition, there was a significant days main effect $F(4,108)=5.72$ $p<0.001$, animals rearing most on day four.

Considering, finally, the two measures often associated with emotionality, number of defecations and seconds spent in the centre of the open field, analysis of variance on these two measures over the five days of testing failed to reveal any significant effects due to the grandmaternal experience $F(2,27)=0.58$ and 0.03 $p>0.05$ for the two measures respectively. Moreover, in neither of the analyses were there any significant interactions $F(8,108)=1.14$ and 0.78 $p>0.05$, for the defecations and time in centre measures respectively, suggesting similar patterns of responding over days for the three groups. Indeed, the only significant effect to emerge in these ANOVAs was due to the days main effect $F(4,108)=2.39$ $p<0.05$, for the time in centre measure, animals spending more time in the centre circle on day three.

c) Skinner Box:

Figure 6:7 describes the learning curves for the three grandoffspring groups over the eighteen days of testing in the in the Skinner box apparatus. Although SEC and SC subjects appear to bar press more than their IC counterparts, analysis of variance of the bar press scores of the groups over all test days proved this to be non-significant statistically $F(2,27)=1.93$ $p>0.05$. Predictably, there was a highly significant days effect $F(13,351)=59.49$ $p<0.001$, but no differences between the groups in rate of learning $F(26,351)=1.21$ $p>0.05$.

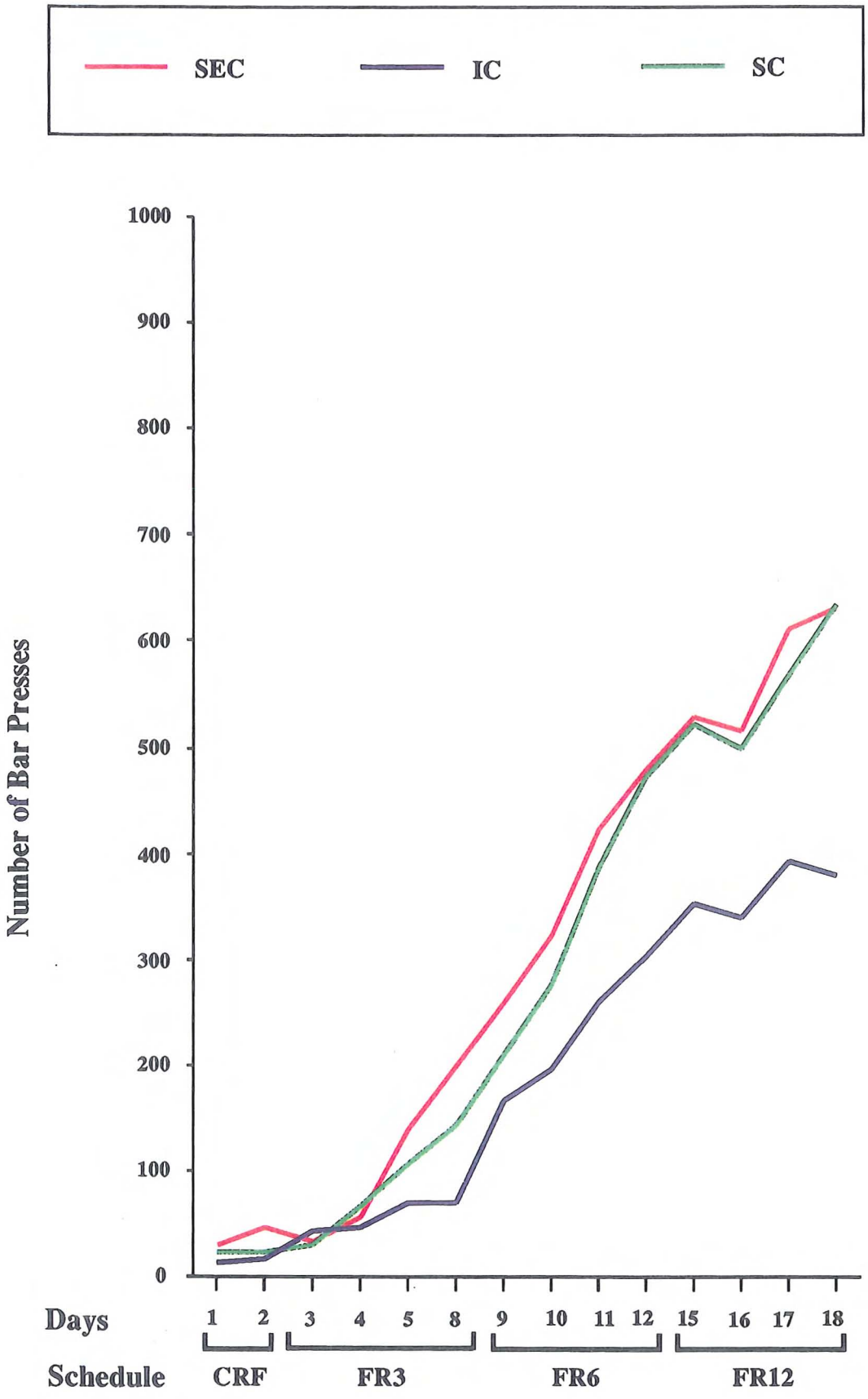


Figure 6:7

Mean number of lines crossed by the three grandoffspring groups over the eighteen days of Skinner box training.

d) Summary of Significant Main Effects:

In the grandoffspring, of the five measures taken (number of lines crossed, number of rears, number of defaecations, time spent in the centre of the open field and number of bar presses in a Skinner box) only one clearly demonstrated significant differences between the groups. This was number of rears. As can be seen from Figure 6:6, grandoffspring of SEC animals reared more than their SC and IC counterparts.

6:3:4 DISCUSSION

The results of this experiment extend the findings of the previous experiment in revealing that exposing female rats to differential environments prior to pregnancy, not only affects their offsprings' behavioural profiles, but can also have a slight effect two generations on. This finding extends the work of Diamond et al (1984), who reported significant increases in cortical thickness in successive generations of offspring of enriched parents, in that it demonstrates a *behavioural* difference between the experimental groups. Furthermore, in the present work, the differences between the grandpup groups were found in a measure of activity, rearing, reminiscent of the activity differences observed in the grandoffspring of animals subjected to avoidance conditioning prior to pregnancy (Wehmer et al 1970) or handling in infancy (Denenberg and Rosenberg 1967). Unlike the latter authors' work, however, which employed fairly stressful and invasive grandmaternal manipulations, the present investigation employed a procedure that McKim and Thompson (1975) have described as a "minimal" stressor, suggesting that even subtle changes in the environmental experiences of an organism can have implications for future generations.

In the present investigation, statistically significant differences only emerged between the experimental groups in the rearing measure, although there was a tendency for SEC and SC grandoffspring to cross more lines and bar press more than their IC counterparts. These results will be discussed in the following pages.

Grandoffspring Weaning Weights:

As with the previous experiment, no differences emerged between the grandoffspring groups with respect to weaning weights. This contrasts with Diamond et al (1984) who reported finding body weight differences in favour of progeny of enriched animals. However, the latter study subjects were weighed at birth, whilst the present results were based on the weights of 21 day old animals, procedural variations which may well account for the differences between the results. In addition, Diamond et al (1984) typically included a period of prenatal enrichment which may have impacted *directly* on the fetuses, further altering the procedural variations between their study and the present work, rendering comparisons difficult. Interestingly, in the present study *grandoffspring* weaning weights averaged 50 grams, a good 10 grams heavier than the weaning weights of *offspring* of animals exposed to SEC, SC and IC. Whether this reflects maturational differences or is just a random fluctuation, is unclear. It should be pointed out, however, that these weight differences between offspring and grandoffspring groups do not reflect differences in littersizes, animals in both experiments coming from similar size litters (see appendices).

In this thesis it has been argued that as a maternal manipulation exposing animals to differential environments should be seen in the context of a minimal stressor. Interestingly, the only other maternal manipulation which has been designated minimal in the prenatal stress literature, namely handling (McKim and Thompson 1975), has also been investigated across two generations. Unlike the present results, however, differences were found in weaning weights of animals whose grandmothers were handled prior to conception, when compared with offspring of control grandmothers (Denenberg and Rosenberg 1967). Specifically, grandoffspring of handled females weighed significantly *less* than those of unmanipulated females. This comparison serves to highlight the importance of the *nature* of the maternal manipulation (as discussed in more detail in chapter three) and suggests that exposing future mothers to SEC, SC and IC which does not have an impact on either their offspring or grandoffspring's weaning weights, cannot be considered a similar type of stressor as handling.

Grandoffspring Open Field Behaviour:

In the rearing measure, SEC subjects were more active than their IC counterparts. Furthermore, there was a tendency for SEC animals to cross more lines than their IC counterparts, although this was not statistically significant. This is in contrast with the results of the previous experiment in which the offspring of IC mothers were more active than their SEC counterparts in both the lines crossed (day one) and the rearing measures. In addition, when comparing the SEC and IC grandoffspring groups with animals exposed directly to SEC, SC and IC (Figures 5:6 and 5:7) grandoffspring performance can be seen as mirror-imaging their grandparent generation's behavioural profiles.

When the SC grandpups performance is included the picture becomes more complex, as their behavioural profile is similar to that of the SEC grandpups in the lines crossed measure and to that of the IC grandpups in the rearing measure. Again this result is in contrast with the SC *offspring* performance, where, for example, SC progeny crossed more lines than the SEC offspring (days one to four), whilst rearing less than the IC offspring and from the grandparent generation, where in the post partum groups, SC dams' performances were statistically indistinct from those of the IC dams.

Unlike either the differentially housed animals or their offspring, however, grandoffspring groups did not differ in terms of the measures typically associated with emotionality, namely time spent in the centre of the open field, and defecation scores. As yet there is no obvious explanation for this finding.

When the results of the present experiment are compared with the two other examples of grandmaternal manipulations in the prenatal stress literature, however, the picture becomes even more confused. Two procedures have been employed as a grandmaternal manipulation, premating avoidance conditioning (Wehmer et al 1970) and handling (Denenberg and Rosenberg 1967). The former manipulation which has been deemed "punitive" by McKim and Thompson (1975)

has been found to have an impact across generations, the latter more "minimal" stressor only having an impact on grandoffspring in conjunction with maternal enrichment. In particular, grandpups of female albino rats which had been subjected to avoidance conditioning before mating were found to be more active in an open field than the descendants of control grandmothers, whilst the effect of handling was only apparent as elevated activity levels in grandoffspring of mothers exposed to enrichment during infancy. If the maternal generation were not given any "free environmental" experience, however, descendants of non-handled grandmothers were found to be more active than the grandpups of handled animals. What seems to be emerging here, is that, as with many other examples of prenatal stress, the nature of the stressor plays an important role in the mediation of the effects across generations. With the more punitive stressor, avoidance conditioning, grandoffspring are more active than their control counterparts. With the more minimal stressor, handling, however, the opposite is true. It is only with the addition of a "top-up" dose of maternal (as opposed to grandmaternal) enrichment that grandoffspring produce higher levels of open field activity. This suggests, in conjunction with the present results where some evidence of grandparent effects also emerged, that exposure to differential environments, unlike handling, has an element within it which produces the effects more commonly associated with a "punitive" stressor, when the effects are investigated two generations further on. The nature of this element is still a matter for conjecture. However, it should be pointed out that if the transfer of effects is being solely mediated by stress, then changes in grandoffspring groups levels of emotionality would also have been predicted. This was not the case, as can be seen from the lack of significant differences between the groups' time in centre and defecation scores. Other factors may also be contributing to the differences in grandoffspring behaviour in an interactive manner. For example, as with the parent generation observed in the last experiment, grandoffspring performances may well also reflect the differentially arousing properties of their mothers' behaviour (these animals in turn altered by their own mothers, Ivinskis and Homewood (1980), coupled with their experiences of being housed in an SC) either from birth, in utero, or both.

Grandoffspring Skinner Box Performance:

Despite a tendency for SEC and SC grandoffspring to bar press more than their IC counterparts (Figure 6:7), this result was not statistically significant. However, it is interesting to note, that yet again the pattern of grandoffspring performances differ from that of both their parent and grandparent generations. For example, animals exposed directly to IC bar pressed more than their SEC counterparts, whilst SC *offspring* bar pressed significantly more than SEC offspring but did not differ significantly from IC offspring. In the grandoffspring groups, a different (albeit nonsignificant) pattern emerged.

Summary and Conclusion:

In this experiment, grandoffspring of animals exposed to differential environments prior to pregnancy were tested in order to investigate their activity and learning performances. Results extended the pioneering work of Diamond et al (1984), in demonstrating some evidence of a behavioural difference between the groups and were reminiscent of the work of researchers employing more stressful maternal manipulations (Denenberg and Rosenberg 1967; Wehmer et al 1970). Furthermore, although only the rearing measure significantly distinguished between the grandoffspring groups, it is of passing interest to note that the grandoffspring performances were fairly consistent across behavioural measures, SEC and SC grandpups being typically more active than their IC counterparts. In view of the lack of statisitically significant data, however, too much must not be made of this observation.

As yet the nature of the mechanisms underlying these effects is not known. However, enrichment has been found to have both behavioural and biological effects on the stimulated organisms and to quote Denenberg and Rosenberg (1967), "these effects could act through changes in the grandmaternal or maternal behaviour or through physiological changes which would affect the developing foetus or modify the milk supply of the grandmother or mother" (p550). Before

attempting to ascertain causality of grandpup effects more rigorously, however, the impact of enrichment on offspring needs to be considered further. Although the fact that enrichment effects can extend across two generations is fascinating, a more valuable avenue of exploration at this point in the present work is to understand the offspring effects before attempting to extend causality across two generations. Consequently, in the next two chapters, the nature of the behavioural differences between offspring of differentially housed mothers provide the main focus of attention and grandoffspring results are left open for future research.

6:4 GENERAL DISCUSSION AND CONCLUSION

The experiments reported in this chapter were designed to investigate the effects of environmental experience on the behaviour of successive generations, an area of research which has received little attention in the EC/IC literature. The results clearly demonstrate that differential housing, as well as affecting the anatomy and behaviour of animals directly exposed to SEC, SC and IC, has a slight effect on behaviour two generations later [17].

Perhaps, not surprisingly, behavioural differences appear to be far stronger in the offspring generation than in the grandoffspring generation and indeed, it is the *nature* of said offspring differences which provide the focus for the next chapter. However, the importance of the present research lies in the fact that it extends the definition of "early experience" beyond the lifetime of one organism. In particular, this present work suggests that early experiential research should not just consider the environmental background that the developing organism has experienced, but must now be aware that the true "nature" of an organism is also influenced in some "non-genetic" (Denenberg and Rosenberg 1967) manner by the environmental experiences of previous generations.

When considering the *nature* of the effects of differential maternal environments, with both the offspring and grandoffspring generations, the patterns are complex. As only one of the five behavioural measures taken in the grandoffspring generation statistically distinguished be-

[17] For a fuller discussion of the relationship between the offspring and grandoffspring effects, the reader is referred to chapter nine, the general discussion of this thesis.

tween the three experimental groups, grandoffspring effects must be treated with some caution. Consequently the remainder of this thesis will focus on the offspring generation where several statistically significant results emerged. More specifically, from the evidence presented in this chapter, offspring of animals exposed to SEC, SC and IC differed in their performances in the open field, Skinner box and to a lesser extent in the visual cliff. The exact nature of these differences, however, remain to be elucidated, as do their causes. It was suggested, however, that these behavioural performances might be mediated by a variety of intervening variables, including endocrine system alteration, neurochemical changes, stress, differential arousal or learning. Of these, the possibility that offspring performances reflect and/or are mediated by different learning capacities, is of considerable theoretical importance and warrants further investigation. Although the present experiment did employ a Skinner box task, as Greenough (1976) points out, the behavioural effects of differential experience seems to be greatest on tasks which are rather similar to a complex environment, such as mazes. Consequently, it was decided to compare the performances of the the three types of offspring in a Hebb-Williams maze. This forms the first experiment of the next study, chapter seven.

CHAPTER SEVEN: THE EFFECTS OF DIFFERENTIAL MATERNAL ENVIRONMENTS PRIOR TO PREGNANCY ON OFFSPRING PERFORMANCE IN THE HEBB-WILLIAMS MAZE AND AN OPERANT CONDITIONING TASK

7:1 GENERAL INTRODUCTION

So far, the experimental work in this thesis has demonstrated clear effects of the environmental manipulation of female rats prior to pregnancy on offspring performance, with slight effects emerging in the grandoffspring generation. The potential role of a variety of factors in mediating the offspring effects were considered in the previous chapter and on the basis of this discussion, it was decided to pursue explanations of a more mechanisitic nature, in particular concentrating on learning, arousal and stress. The first experiment in this chapter examines these explanations further, with specific attention to learning, as measured by offspring performance in the Hebb-Williams maze.

7:2 EXPERIMENT ONE

7:2:1 INTRODUCTION

According to Renner and Rosenzweig (1987) "in studying the effects of differential environments on brain and behaviour, the obvious implication from findings that animals with an enriched history exceed their impoverished counterparts in many brain measures is that they will also be behaviourally superior" (p40). Although it is now apparent that EC animals are behaviourally *different* from their IC counterparts (see chapter two) there is some debate as to whether these differences reflect enhanced learning capacity per se in the EC animal (Renner and Rosenzweig 1987), deficiencies in the IC animal (Dell and Rose 1986) or even EC/IC differences in non-learning functions (see chapter two). Nowhere is this debate more obvious than in the maze-learning literature and in particular, in the analyses of the performances of differentially housed animals in the Hebb-Williams maze.

This paradigm, first described in 1946 as a method of rating animal intelligence, has been extensively employed in the EC/IC literature, with numerous authors reporting superior performance in animals raised in complex environments when compared with animals raised in isolation or in

socially housed conditions (see chapter two). Although at first this performance difference was interpreted as a quantitative difference in intelligence between the experimental groups (Hebb and Thompson 1954; Hebb 1947; 1949) what is now clear is that enrichment produces an animal that is qualitatively different from its IC or SC counterpart.

More specifically, EC animals are better able to use extra-maze cues when successfully negotiating the Hebb-Williams maze (Forgays and Forgays 1952; Ravizza and Herschberger 1966) whereas isolation induces higher levels of exploration resulting in increased error scores (Zimbardo and Montgomery 1957; Woods 1959; Woods et al 1960; 1961). This has lead some researchers to conclude that the IC animal fails to habituate to irrelevant diversions in the maze pathways thus maintaining their higher error scores over trials (Dell and Rose 1986). However, according to Renner and Rosenzweig (1987), deficiency in response inhibition in the IC animal cannot account for all the problem solving differences observed in the EC/IC literature (reviewed in chapter two) and they argue that "no explanation of the existing behavioural differences documented thus far can account for the variety of findings without including some type of cognitive difference between EC and IC subjects" (p48).

If, as these authors suggest, EC and IC animals have different environmentally induced cognitive capacities, then an important question for this present work is, simply, do offspring of differentially housed mothers have different cognitive capacities as well? The purpose of the present experiment was to explore this further by examining offspring performance in the Hebb-Williams maze.

In reviewing the available learning paradigms that might be appropriate for testing the performance of offspring of differentially housed mothers, Greenough's (1976) contention that behavioural effects of differential experience seem to be greatest in maze tasks was taken into account. Beyond that, the choice of Hebb-Williams as opposed to the other types of mazes available was made for several pertinent reasons.

Firstly, as has been noted, there is already an extensive literature [1] detailing the performances

[1] Albeit not an entirely consistent one.

of animals exposed *directly* to EC, IC and SC in this apparatus against which to compare those of the offspring. This allows for conceptual links to be drawn between the offspring and parent generations. Secondly, in a fairly recent publication Dell and Rose (1986) have modified the more traditional Hebb-Williams maze by extending the height of the walls and barriers to make it an open rather than closed field. This methodological alteration allows the experimenter to monitor error scores (the behavioural measure associated with problem solving ability), number of squares entered (a measure of activity) and number of rears (according to these authors a measure of exploration) separately, thus offering a more comprehensive account of the animals' Hebb-Williams performance. This methodology might therefore be useful in isolating causal differences between the offspring.

The final reason for choosing the Hebb-Williams maze was that, of those few studies investigating the effects of enrichment of the offspring during the postpartum period [2] on their learning performance or, of more relevance to the present work, enrichment of the mother *during* pregnancy on their offsprings' learning performance [3], have all employed the Hebb-Williams maze (see Table 1:1, chapter one). This is a precedent unrivalled by any of the other maze paradigms. To date, however, it should be noted that no experiments have employed this task to investigate the effects of animals exposed to differential environments *prior to pregnancy* on their offspring, a time when the environmental experience could not possibly affect the offspring directly, but only in a manner mediated through the maternal response.

Although postpartum enrichment has been found to have an impact on the offsprings' Hebb-Williams performance, with for example the most recent publication employing this paradigm (Muir et al 1985) reporting that pups subjected to enrichment from birth to day seven made fewer errors in the Hebb-Williams maze than control groups when tested at either 65 or 110 days old, this procedure does not separate out the impact of *direct* enrichment of the offspring

[2] Dawson and Hoffman 1958; Forgays and Read 1962; Schwartz 1964; Smith 1972; Will et al 1976; Muir et al 1985; Venable et al 1988

[3] Ravizza and Herschberger 1966; Denenberg, Woodcock and Rosenberg 1968; Ivinskis and Homewood 1980; Kiyono, Seo and Shibagaki 1982; Kiyono, Seo, Shibagaki and Inouye 1985.

from that engendered by any maternal influence. This criticism can also be leveled at three of the "prenatal" studies (Ravizza and Herschberger 1966; Denenberg et al 1968; Ivinskis and Homewood 1980) in that they too, included a degree of postnatal enrichment. However, these studies where enrichment of the mother during pregnancy do have one thing in common with the present work, namely that for a period of their life (in utero) these offspring can only have been affected by the differential environments via their mothers, consequently they will be reviewed in some detail, to ascertain whether these procedures do have an effect, against which to compare offspring of mothers exposed to SEC, SC and IC prior to pregnancy, which were the subjects of this study.

The earliest study [4] to expose pregnant animals to a "free environment" (Ravizza and Herschberger 1966) placed female rats individually into cages that afforded them extensive climbing experience five days prior to the birth of their offspring. Control animals were housed in environments that restricted motor experience. Offspring were maintained in these environments postpartum until they were weaned at which time they were housed individually in cages identical to the ones in which they were born. When tested as adults, offspring of mothers given access to motor experience exhibited more activity in table top exploration and, of relevance to the present experiment, "showed superior performance in the Hebb-Williams intelligence test" (p73).

Prenatal enrichment in the more traditional sense (ie: social housing with toys) was first employed by Denenberg et al (1968). Their subjects were female rats born to mothers which had been handled in their own infancy and which were placed into either maternity cages or free environments whilst pregnant. Offspring were raised in these environments and once weaned were reared in either SC (N=2 to 3) or EC (N=10 to 12) until 50 days old. When tested in adulthood (370 days) both the preweaning and postweaning experiences emerged as significant main effects, reducing number of errors in the maze. Admittedly the EC experience after wean-

[4] None of these studies have been reviewed in detail although their main findings are described in Table 1:1, chapter one.

ing had a greater impact on Hebb-Williams performance than the preweaning experience, but as the authors point out "the surprising finding is that preweaning enriched experience had such a positive effect" (p535).

Their surprise reflected the generally held notion at that time, that enrichment must occur after weaning and before maturity in order to produce the most pronounced changes in problem solving (Hymovitch 1952). More specifically, the optimum postweaning period for enriched stimulation in rats was considerd to be between 22 to 43 days (Forgays and Reid 1962) and between 50 to 60 days (Nyman 1967), with Denenberg (1966) even suggesting that learning plays only a minor role in preweaning stimulation. Ever cautious, Denenberg et al (1968) concluded that "because of the extreme immaturity of the rat at birth, it is likely that enrichment during infancy only has an effect upon the rat during the last week to 10 days of the preweaning period, ie., after it has developed proficiency in locomotion and after the eyes have opened" (p535). This conclusion, however, has proved to be erroneous as exposure to enrichment between days 0 to 7 (a time when rats' eyes are still closed, and locomotion is minimal) has since been found to reduce error scores when compared with control animals (Ivinskis and Homewood 1980).

This latter experiment and those of the Japanese researchers (Kiyono et al 1982; 1985) are unique in the EC/IC literature in that they have tested the effects of enrichment of the mother (during pregnancy) on offspring problem solving ability [5], making sure that (like the present thesis) the offspring themselves are not exposed to direct environmental experience. That is, any effects must be mediated by the mother.

As with many areas of research in the EC/IC literature, these three studies have produced conflicting results, two (Kiyono et al 1982; 1985) reporting facilitative effects of prenatal enrichment in postnatal Hebb Williams performance, the remaining study (Ivinskis and Homewood 1980) concluding that "the benefits that the mother might have derived during such an exposure were not transmitted to the fetus. It seems that for significant improvement to occur in offspring,

[5] Ivinskis and Homewood employed two separate procedures, either stressing dams prenatally or post partum

behavioural environmental stimulation must be provided during the postnatal period" (p339). Procedural variations may well partially account for the discrepancy in results. For example Ivinskis and Homewood maintained their animals in EC and SC for the last trimester of pregnancy, whereas Kiyono and his colleagues maintained their groups in differential environments for the whole gestation period. One fact, however, stands out, namely Ivinskis and Homewood compared EC offspring with SC controls, whilst Kiyono et al (1982; 1985) employed EC, SC and IC offspring. In their 1985 paper, these latter authors reported only finding significant differences between EC and IC animals, that is the EC versus SC differences were not significant. This suggests that Ivinskis and Homewood's conclusion was a touch premature and that prenatal enrichment can have a beneficial effect on offspring Hebb Williams problem solving behaviour, when compared with an appropriate control group.

To summarise these findings and place them in context, it can be seen that prenatal enrichment whether coupled with, or independant from postnatal enrichment, can have a positive effect on offspring's problem solving ability. Furthermore, although changes in behaviour produced by environmental stimulation during the preweaning period have usually been explained in terms of direct alterations of the neuroendocrine mechanisms which behaviourally are manifested in lower levels of emotionality (Denenberg and Zarrow 1971, see also chapter three for review of this literature) there are other ways of looking at this issue.

Ivinskis and Homewood (1980) have argued that these effects might be mediated by the mother "an important agent in supplying stimulation and maintaining the arousal of the infant" (p337) which, as has been proposed by Walsh and Cummins (1975), mediates the effects of enrichment. They suggest "it is therefore possible that, through this mother-infant relationship the beneficial effects of the enriched environment can be transmitted to the infant rat during the early part of the preweaning period" (p337). This suggestion is consistent with some of the findings of the present thesis, and has provided one possible explanation for the offspring effects reported in the previous chapter. However, as noted earlier, arousal is but one possible cause of the prior

to pregnancy induced effects. Learning differences between the offspring groups, engendered by their experiences with qualitatively different mothers have also been postulated as causing the offspring differences.

The purpose of the present experiment was to investigate the nature of offspring differences further, using a paradigm that tests for problem solving ability, the Hebb-Williams maze. This paradigm has several advantages, in that it can (following the procedure developed by Dell and Rose 1986) isolate activity, exploration and learning, in as much as these variables can be differentiated from each other, thus offering a way of exploring both offspring learning capacity and the postulated causes of the offspring differences.

More specifically, if, as has been suggested, SEC mothers are in some way altering the learning capacity of their offpsring, then SEC pups should quite simply make fewer errors than the SC and IC offspring in the various mazes. Furthermore, their learning curves, as measured by number of errors over the eight trials in the six mazes employed, should show a faster reduction of errors over trials than that of their SC and IC counterparts. If on the other hand, offspring groups are differentially stressed or aroused by their experiences with their mothers, then differences should emerge between the groups in measures of emotionality and activity [6].

One final decision was also taken in this experiment which should be explained. As with the grand offspring study (see previous chapter) only male offspring were employed in this experiment. This was for two reasons: firstly, the procedure to be adopted in the present study was developed using male animals (exposed directly to enrichment) and it is their behavioural profiles that are being used as a standard against which to compare offspring profiles, so it was deemed appropriate to employ male animals in this study. Secondly, the Japanese researchers who reported differences in Hebb-Williams performances in animals exposed to enrichment and impoverishment whilst in utero (Kiyono et al 1985), also used male rats, further justifying this decision. It should be noted,

[6] Obviously differing levels of activity and emotionality in the offspring may reflect either differential stress levels or arousal levels in these animals. In order to seperate out these factors, arousal levels are investigated further in the next chapter.

however, that earlier studies using the closed field version of the Hebb-Williams have employed female animals (Denenberg et al 1968; Kiyono et al 1982) so there is a relevant female baseline available that could be employed in future research.

7:2:2 METHODOLOGY

a) Subjects:

These were 39 male F2 generation Hooded Lister rats of weanling age (21 days), 13 animals being bred from F1 generation females exposed to either SEC, SC or IC prior to pregnancy, following the procedure oulined in the general methodology chapter (chapter four). Because of the length of time the Hebb-Williams procedure takes on any one day, it was decided to breed the animals in two batches, care being taken to ensure that consistency was maintained in the procedures employed for the two groups. Seven offspring were obtained from each maternal condition in batch A, six in batch B, equal numbers of litters being represented in each case.

b) Apparatus:

Based upon the modification developed by Dell and Rose (1986), the Hebb-Williams apparatus employed in this experiment is described in detail in the general methodology chapter (chapter four).

c) Procedure:

At weaning subjects were weighed and assigned to individual cages for a day prior to the start of the experimental procedure. During this time, all the animals from SEC, SC and IC mothers were recoded by a technician so that the experimenter was unaware of each animals' background, thus removing any confounding expectancy effects (Rosenthall 1966). Starting at 22 days of age,

subjects were put through the following maze training and testing phases.

PHASE ONE: PRE-TRAINING PERIOD: This consisted of habituating the animals to the experimental apparatus. In particular, on days one and two, all animals were placed in the goal box of the maze (see Diagram 7:1) with a bowl containing five Noyes pellets. Animals were left in the goal box until they had either eaten all the pellets, or five minutes had expired. Time to eat all the food was recorded, animals not eating the pellets being given a score of 300 seconds (five minutes). On days three, four and five, animals were placed in the start box of the Hebb-Williams maze and trained along a runway connecting start and goal boxes. Again, animals were removed once they had eaten all the pellets in the goal box, or after five minuteds had elapsed. In this part of the procedure, latency to leave the start box and time to reach the goal box was measured. For these first five days of training, animals were given two trials a day.

PHASE TWO: TRAINING PERIOD: This consisted of training the animals using a procedure based on that of Rabinovitch and Rosvold (1951). Subjects were trained on six practice mazes (see diagram 7:2) to a criterion of nine correct solutions [7] in less than 60 seconds on two consecutive days. Animals reaching this criterion early, as recommended by Rabinovitch and Rosvold, were given three trials per day until all animals had reached criterion. Consequently, all the animals were exposed to all of the practice mazes, albeit for varying number of trials, animals having reached criterion early only receiving three trials per maze rather than nine. In this part of the training procedure, animals' latency to leave the start box, number of squares entered in the maze and time to reach the goal box were recorded. Number of days to reach criterion for each animal was also calculated.

PHASE THREE: TESTING PERIOD: This consisted of testing all the subjects on six maze problems (Numbers 1, 3, 5, 7, 9 and 11 of Rabinovitch and Rosvold's (1951) 12 test mazes: see Diagram 7:3). The problems were presented on consecutive days, subjects being given eight trials on each one. Latency to leave the start box, time to reach the goal box, total time (that is the

[7] A correct solution consisted of the animal reaching the goal box, where it was fed five Noyes pellets.

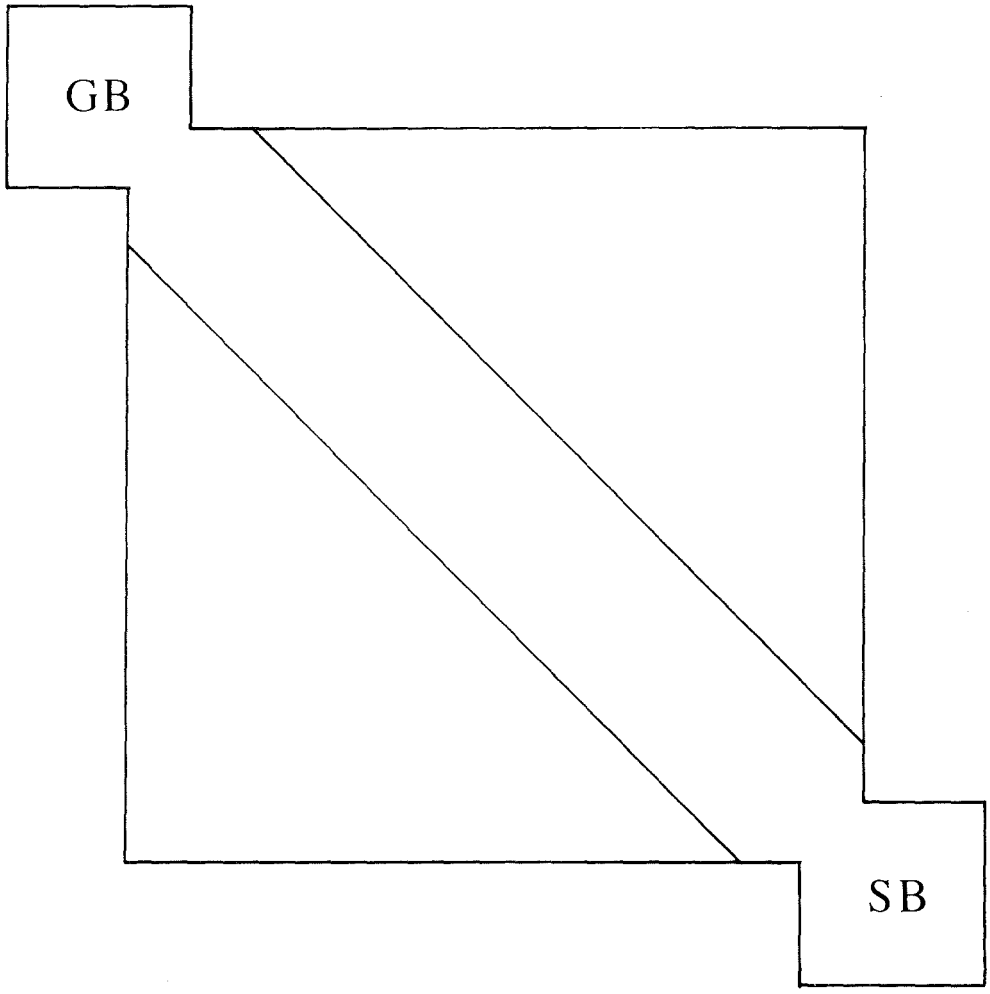


Diagram 7:1
Schematic representation of positioning of Runway
within the maze apparatus employed in the pre-training period.

Bold Lines = Barriers
SB = Start Box; GB = Goal Box

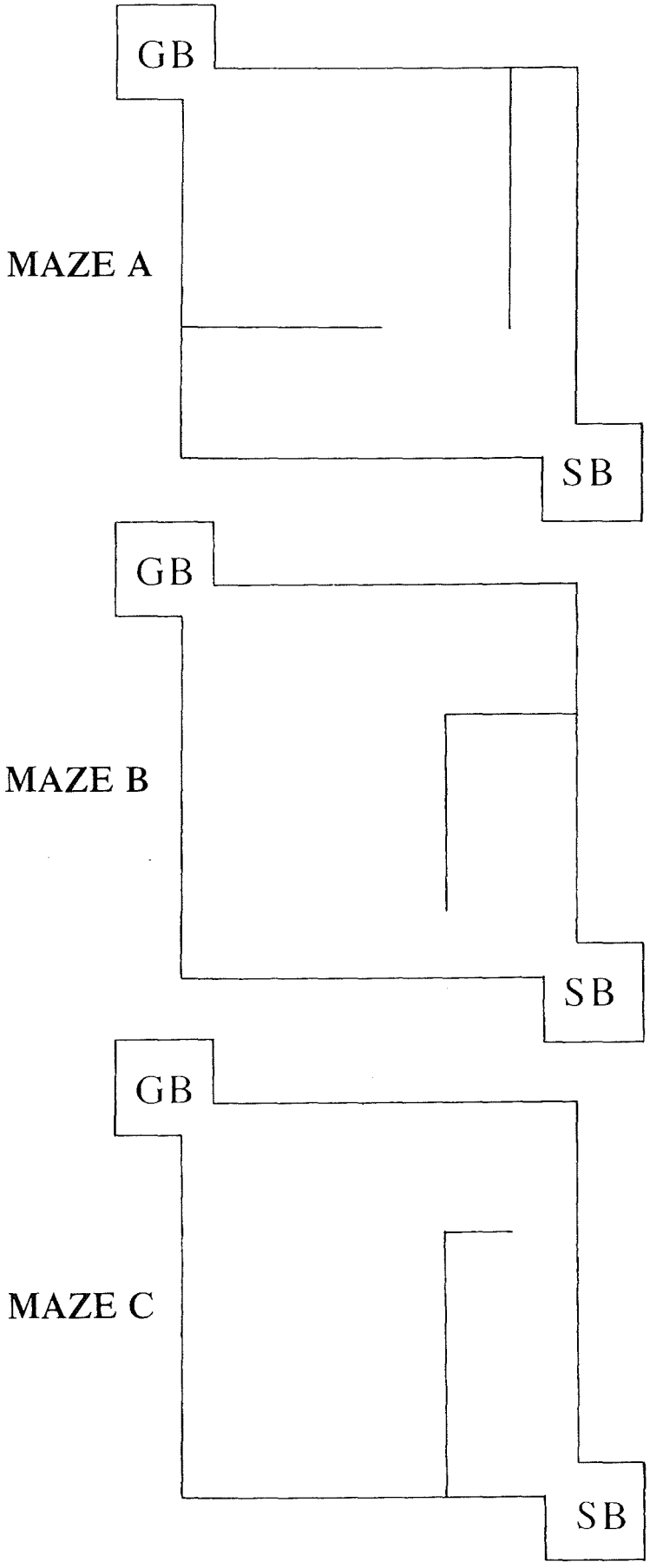


Diagram 7:2
Schematic representation of the six practice mazes employed in this thesis based on those employed by Rabinovitch and Rosvold (1951)

Bold Lines = Barriers
SB = Start Box; GB = Goal Box

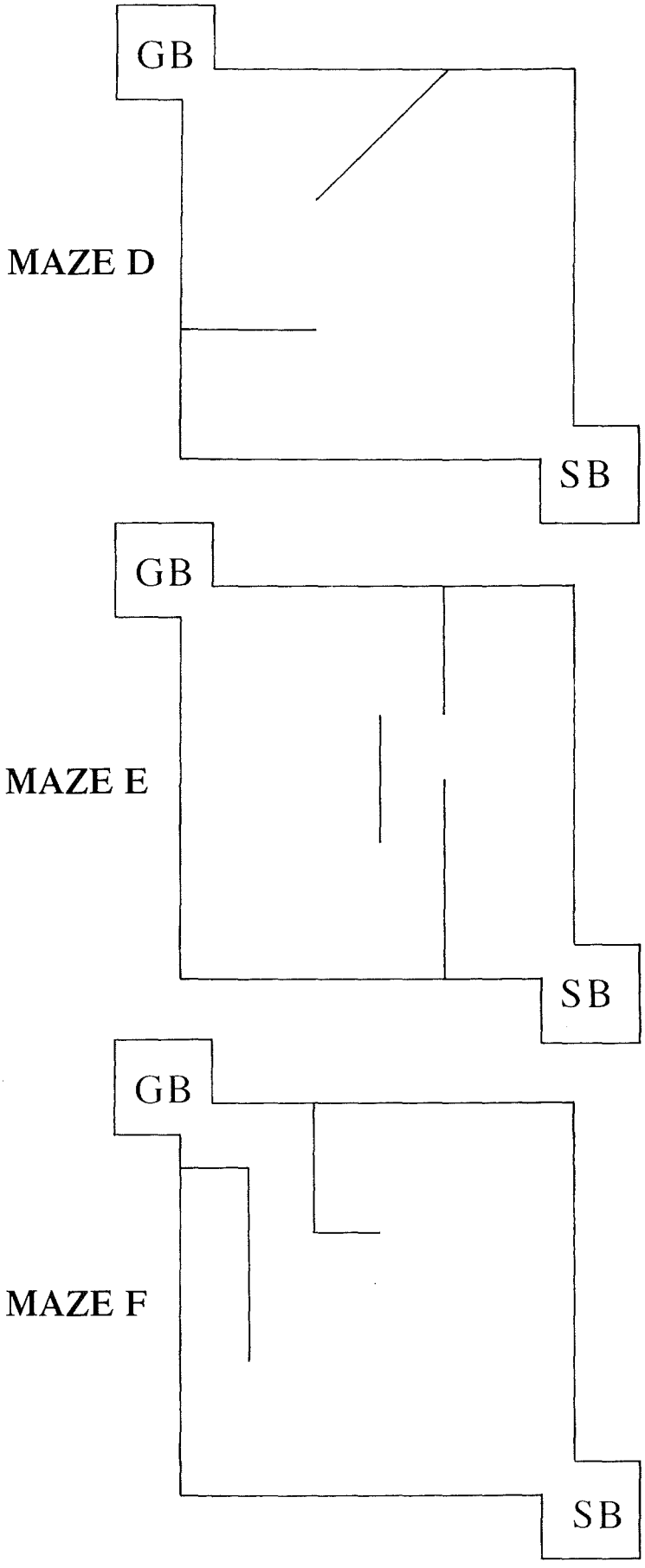


Diagram 7:2 Continued

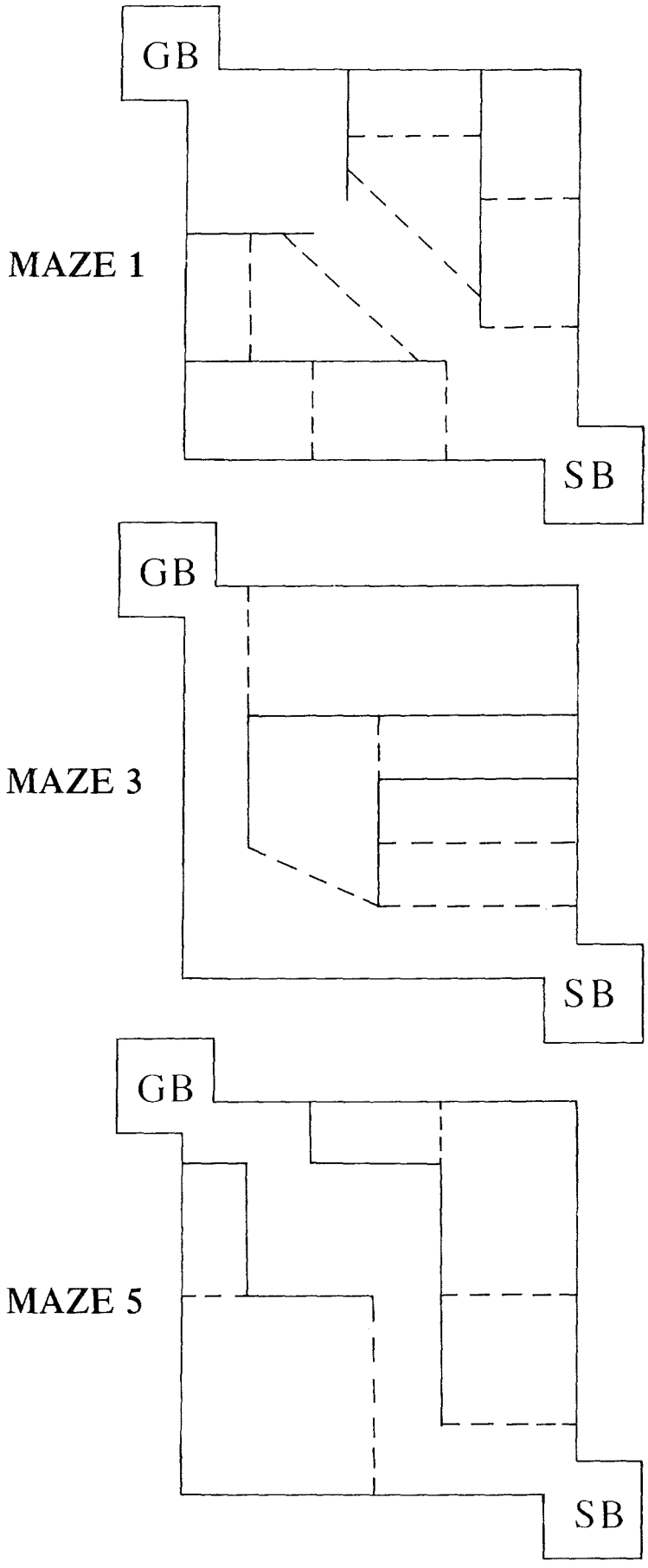


Diagram 7:3
Schematic representation of the six test mazes employed in this thesis, taken from the 12 test mazes designed by Rabinovitch and Rosvold (1951)

Bold Lines = Barriers Dotted Lines = Error Zones
SB = Start Box; GB = Goal Box

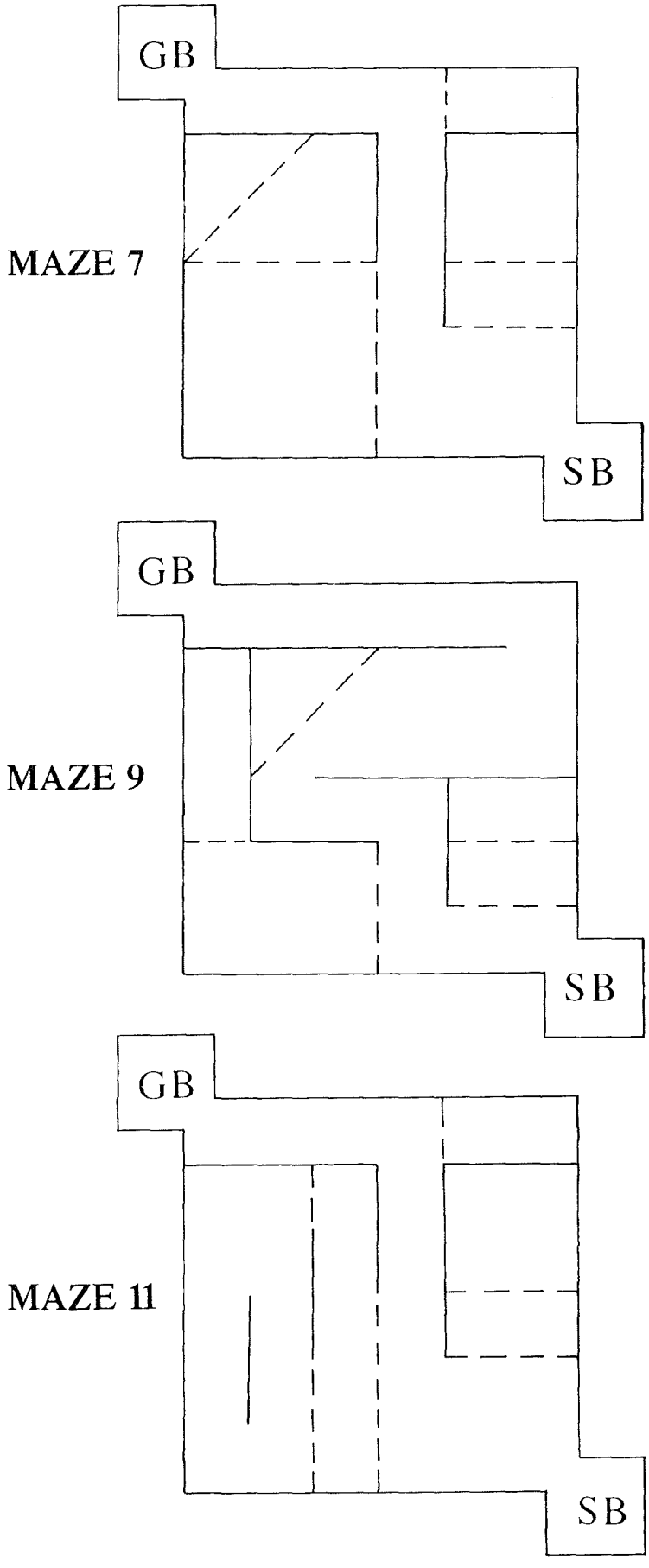


Diagram 7:3 Continued

time from being placed in the start box until all the food was eaten), number of retraces (that is number of times the animals re-entered the maze from the goal box), numbers of initial and repetitive errors [8] (those that the animals made on re-entering the maze from the goal box), squares entered and rears were recorded. Animals were fed five Noyes pellets in the goal box in each trial.

At all stages of testing, animals were given a maintenance diet of eight grams of breeding diet at the end of each day and were weighed each morning to ensure that the food deprivation schedule was not adversely affecting their normal growth patterns [9].

7:2:3 RESULTS

As with the previous experiment (chapter six) littersize effects were checked using a one way analysis of variance, with size of litter from which each subject was drawn as the dependant variable. Results indicated that there were no significant variations between experimental groups with respect to littersize $F(2,36)=0.18$ $p>0.05$, consequently individual animals were used as the unit of analysis [10].

Data from both the pre-training period (phase one) and training period (phase two) were analysed, as were measures taken during the testing period itself. These will be reported in the next two subsections.

PHASES ONE AND TWO: PRE- TRAINING AND TRAINING

In this section, measures taken from both the pre-training and training phases of the experiment will be described. Considering the initial pre-training procedure first, animals were habituated

[8] An error is defined by the animal crossing into an error zone with its two front paws, error zones being described by dotted lines in Diagram 7:3.

[9] Growth rate curves for Hooded Lister rats having been supplied by Harlan-Olac Ltd.

[10] As before, it should be noted that this procedure does not eliminate the possibility that different littersizes might be contributing to differences in individual animal's behaviour. What this procedure does insure, however, is that littersize variations are equally distributed across the three experimental groups.

to the experimental apparatus by being fed in the goal box. Latency to eat all the Noyes pellets was recorded over the four trials and revealed a significant difference between the experimental groups $F(2,36)=3.66$ $p<0.05$, which when examined more carefully by post hoc Newman Keuls, revealed that SC offspring consistently took longer to eat than their SEC (Newman Keuls $p<0.05$) counterparts. A significant trials effect also emerged, $F(3,108)=59.43$ $p<0.001$, animals taking less time to eat their food over the four trials (mean latency to eat food in seconds: Trial one=276.7; Trial two=194.7; Trial three=132.8; Trial four=105.7) [11]. No other effects were significant (see appendix for ANOVA tables for all analyses reported in this experiment).

Moving on to the second phase of pre-training, animals were required to leave the startbox, run down an alley and enter the goal box, where their food was waiting for them. Time to leave the start box was measured (in seconds), as was time to reach the goal box for the six trials. Neither of these measures differentiated between the three experimental groups $F(2,36)=2.49$ and 2.33, $p>0.05$ respectively. Significant trial effects did emerge for both measures, however, $F(5,180)=3.68$ $p<0.003$ and 19.56 $p<0.001$ respectively. Although no consistent pattern emerged over trials with respect to the the latency to emerge measure, apart from the last trial, animals tended to take less time to reach the goal box on subsequent trials. No significant group by trial interactions emerged for either measure $F(10,180)=1.21$ and 1.04 $p>0.05$, suggesting that the groups patterns of responding were statistically similar over trials.

In the final training phase, animals were trained for nine trials a day on one of the six practice mazes as recommended by Rabinovitch and Rosvold (1951). Animals reaching criterion early were given three trials a day until testing began for all the animals. One obvious learning measure, therefore, was whether the three offspring groups took different numbers of training days to arrive at criterion. As can be seen from Table 7:1, IC animals on average took more days to reach criterion than either their SC or SEC counterparts. However, this result was only statistically significant at the 8% level $F(2,36)=2.775$ $p<0.08$, a result which should be treated

[11] As can be seen from the means of the three offspring groups reported in the appendix, for the last trial (four) offspring of SEC animals took considerably less time to eat than the other two groups. However, this was not statistically significant.

with caution.

OFFSPRING GROUP	DAYS TO CRITERION
SEC	4.77
SC	6.85
IC	7.15

Table 7:1 Average number of days taken to reach criterion, for the three offspring groups.

As some animals in the SEC group achieved the first part of the "criterion" in the second day of training (that is, had completed nine trials in less than a minute) only the results of the first day of maze training were analysed in any detail. As noted in the methodology section, two measures were taken throughout this phase of training, number of squares entered on the way through the maze (no error scores are recorded in this part of training) and time to reach the goal box (in seconds). Analyses of variance of the first of these two measures, number of squares entered, revealed no differences in activity between the three groups $F(2,36)=2.35$ $p>0.05$ although there was a significant trials effect $F=(8,288)=11.36$ $p<0.001$, animals reducing their activity over trials. This pattern of responding was similar for the three offspring groups, as evidenced by a lack of significant group by trials interaction $F(16,288)=0.65$ $p>0.05$, which can be clearly seen in Figure 7:1.

Interestingly, however, the second measure taken, time to reach the goal box, did differentiate between the three offspring groups. Analysis of variance of the three experimental groups' latency to arrive at the goal box over the nine trials on the first day of testing revealed a significant groups' effect $F(2,36)=5.64$ $p<0.007$, which was due to the SC and IC offspring taking longer to reach the goal box than their SEC counterparts (Newman Keuls $p<0.01$). This can be seen from Figure 7:2, which details the amount of time each of the groups took to go through the maze over the nine trials. In addition, there was a significant trials effect $F(8,288)=11.56$ $p<0.001$, animals taking longer in the initial trials than the later ones, as would be expected. This pattern of responding was similar for the three groups, as there was no significant trials by group main

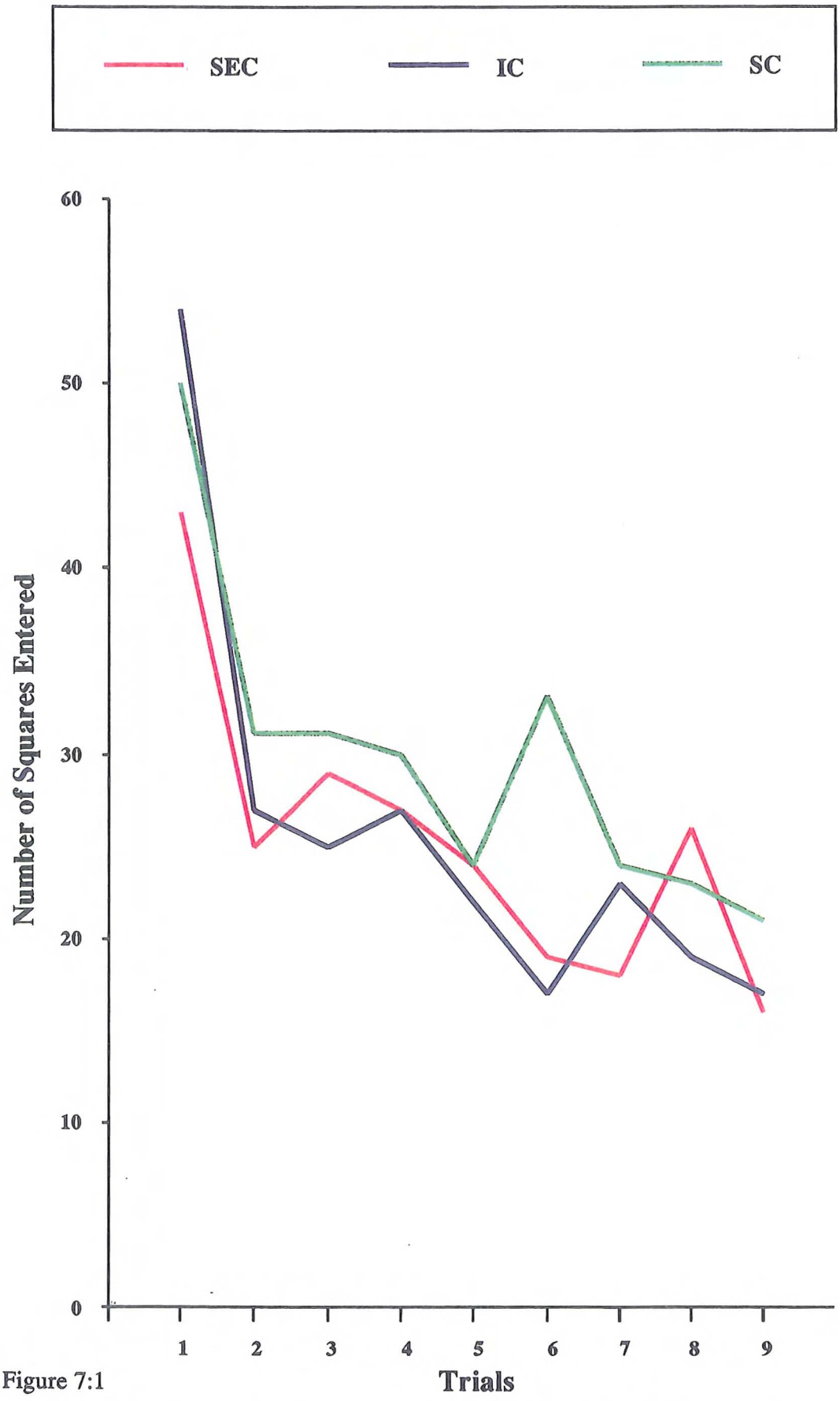


Figure 7:1

Mean number of squares entered by the three offspring groups over nine trials, on the first day of maze training, Practice Maze A. Key: SEC=offspring of SEC dams, SC=offspring of SC dams, IC=offspring of IC dams for all Figures in this chapter.

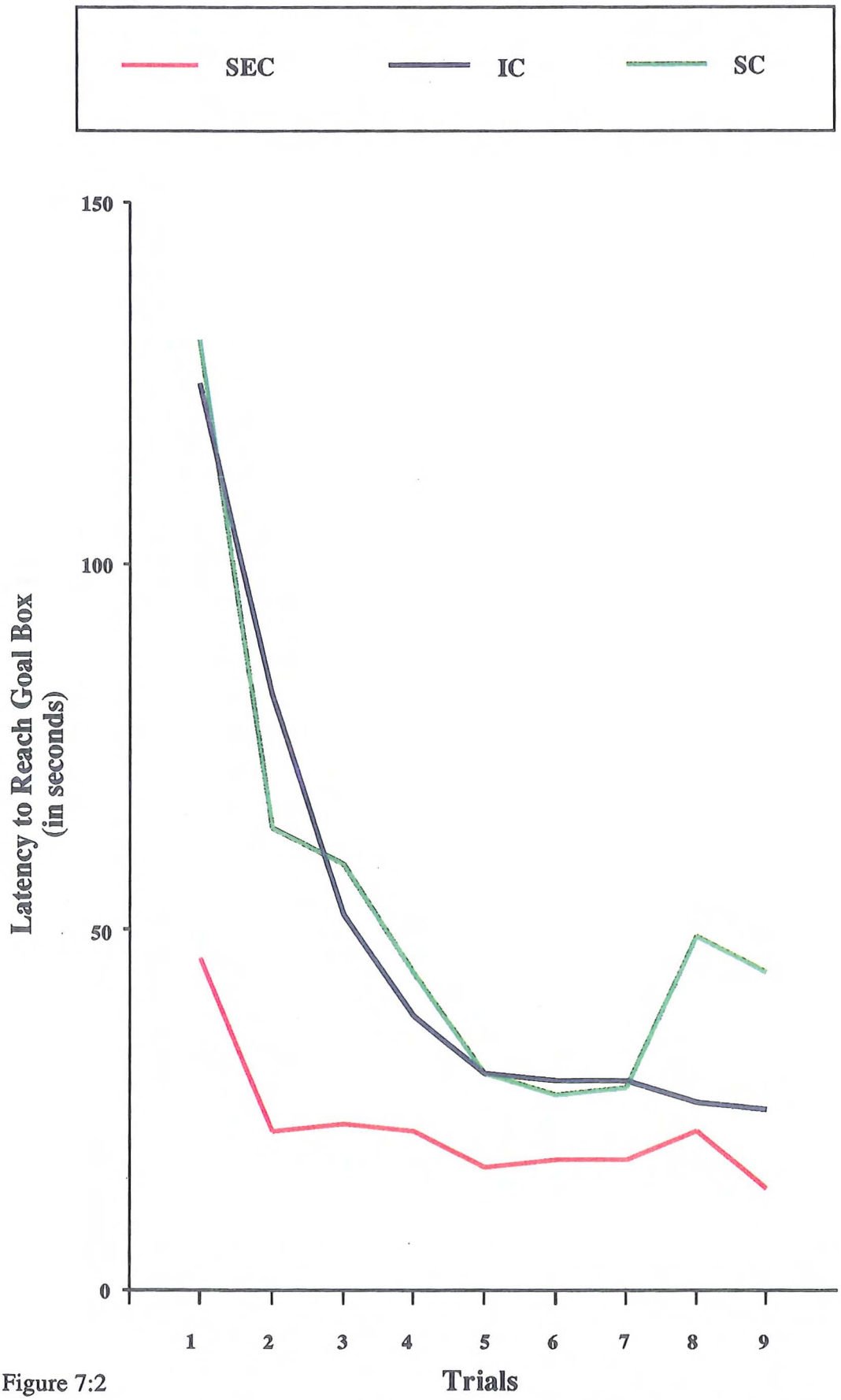


Figure 7:2

Mean time taken to reach the goal box (in seconds) by the three offspring groups over the nine trials on the first day of maze training, Practice Maze A.

effect $F(16,288)=1.45$ $p>0.05$ despite the SC progeny's performance on the last two trials.

PHASE THREE: TESTING

In this section, analyses of the measures taken during the final phase of maze performance, the testing phase are described. For three of the measures (errors, squares entered and rears) initial and retraced errors were recorded. However, as so few animals re-entered the maze after reaching the goal box over the six test mazes, with no significant differences emerging between the three experimental groups on number of re-traces over all the maze problems $F(2,36)=0.95$ $p>0.05$, only the total scores (initial and retraced combined) on each of these measures will be reported.

a) Number of Errors:

An analysis of variance of the total numbers of errors that each animal made on each of the eight trials of the six test problems revealed no significant differences between the three experimental groups $F(2,36)=0.99$ $p>0.05$. Furthermore, although there was an expected trials effect as the animals learnt each new maze $F(7,252)=27.86$ $p<0.001$, no significant experimental group by trials interaction emerged $F(14,252)=0.72$ $p>0.05$. This is clearly evident from Figure 7:3, in which the mean number of errors for the three experimental groups, over the eight trials, collapsed over the six mazes are described. As can be seen from this graph, all three groups gradually reduced their errors over trials following generally similar patterns. Interestingly, there was a days effect, in that some test mazes were more difficult than others as evidenced by the greater number of errors made by all the animals $F(5,180)=18.33$ $p<0.001$, (mean number of errors per maze: Maze 1: 1.22; Maze 3: 2.35; Maze 5: 3.92; Maze 7: 2.82; Maze 9: 2.76 and Maze 11: 2.83), but again this did not separate out the groups, as revealed by the lack of a significant group by mazes interaction $F(10,180)=1.19$ $p>0.05$ described in Table 7:2.

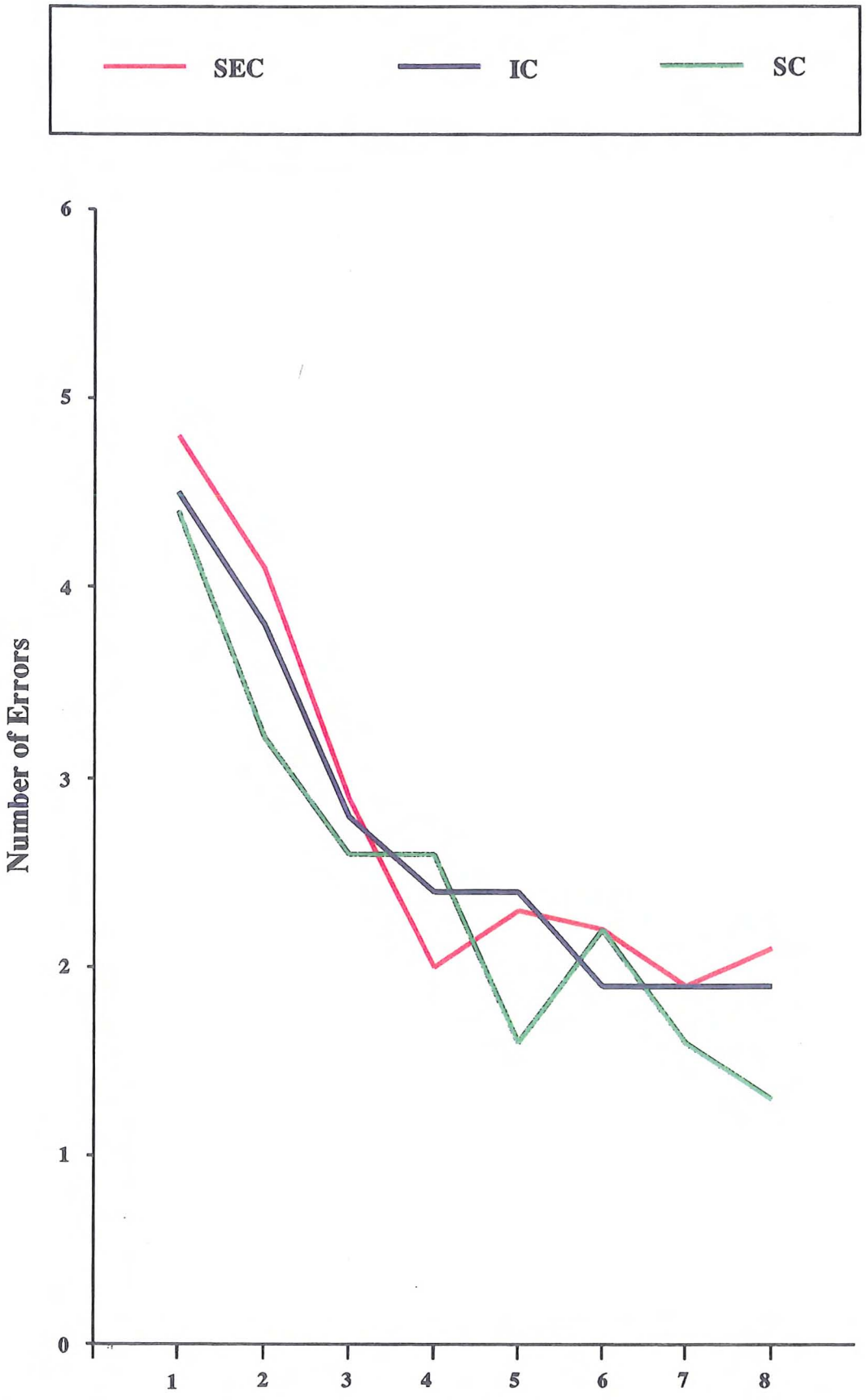


Figure 7:3

Mean number of errors made by the three offspring groups over eight trials, scores collapsed over the six test mazes.

GROUP	MAZE 1	MAZE 3	MAZE 5	MAZE 7	MAZE 9	MAZE 11
SEC	1.01	2.38	4.41	3.49	2.54	2.99
SC	1.32	2.13	3.70	2.60	2.44	2.38
IC	1.35	2.52	3.64	2.38	3.31	3.11

Table 7:2 Means of number of errors made by the three offspring groups over the six test mazes, collapsed over the eight trials.

Finally, there was a significant trials by maze effect $F(35,1260)=1.68$ $p<0.008$ confirming that on those mazes where more errors were scored, the pattern of errors remained higher than in those mazes where fewer errors were scored. This can be seen clearly in Table 7:3, which details the number of errors per trial of all the animals, irrespective of experimental background, over the six different test problems.

MAZE	TRIAL 1	TRIAL 2	TRIAL 3	TRIAL 4	TRIAL 5	TRIAL 6	TRIAL 7	TRIAL 8
1	3.56	1.56	0.77	0.82	0.85	0.51	0.74	0.97
3	6.00	3.44	2.64	1.77	1.67	1.31	1.05	0.90
5	6.23	5.95	3.97	3.49	2.77	3.62	2.82	2.51
7	4.36	3.41	2.95	2.67	2.33	3.00	1.97	1.90
9	3.82	3.4	3.00	2.38	2.33	2.33	2.51	2.28
11	3.49	4.46	3.15	2.97	2.62	1.97	1.74	2.21

Table 7:3 Mean number of errors scored by all the animals over the six test mazes, describing the progression of errors over trials.

No significant trials by maze by group interaction emerged $F(70,1260)=1.09$ $p>0.05$, demonstrating similar patterns of responding over trial and mazes for the three offspring groups.

b) Number of Rears:

A three way ANOVA of total number of rears of the three experimental groups' performance on each of the eight trials per six test mazes revealed no significant differences between the groups $F(2,36)=0.64$ $p>0.05$, nor, as can be seen from Figure 7:4, was there a significant trials by group interaction $F(14,252)=0.79$ $p>0.05$, although there was a significant trials effect $F(7,252)=4.59$ $p<0.001$. This latter result is not unexpected, animals tending to rear more on initial trials

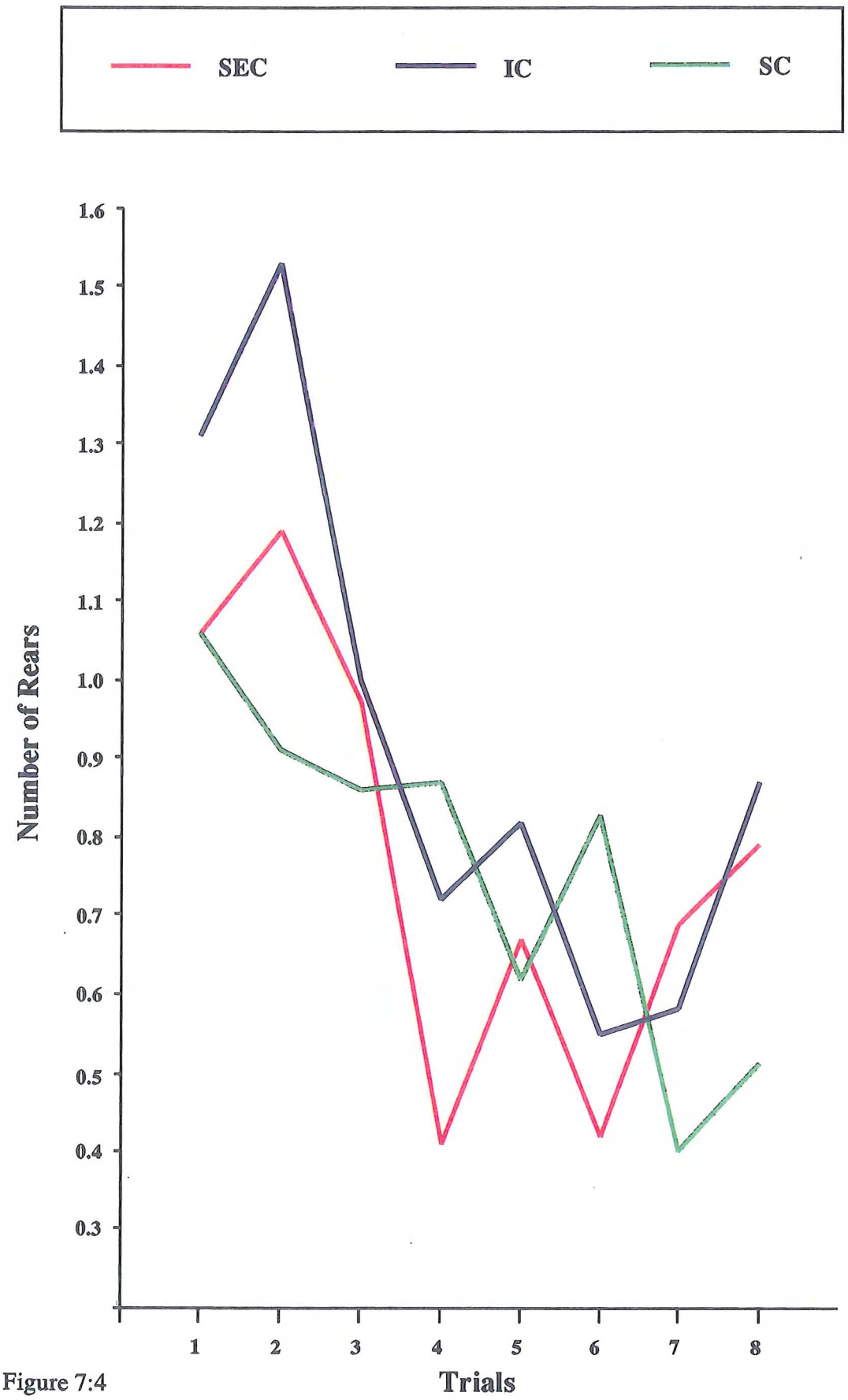


Figure 7:4

Mean number of rears by the three offspring groups over eight trials, scores collapsed over the six test mazes.

whilst learning the maze, than on the later trials (mean number of rears per trial collapsed over the six test mazes: Trial 1: 1.15; Trial 2: 1.21; Trial 3: 0.94; Trial 4: 0.67; Trial 5: 0.70; Trial 6: 0.60; Trial 7: 0.56 and Trial 8: 0.73). As with the error scores, some test mazes elicited more rears than others (mean number of rears per maze: Maze 1: 0.42; Maze 3: 0.57; Maze 5: 1.09; Maze 7: 0.62; Maze 9: 1.03 and Maze 11: 1.19) as evidenced by a significant mazes effect $F(5,180)=6.20$ $p<0.001$, but this did not distinguish between the three groups $F(10,180)=0.38$ $p>0.05$, all demonstrating similar patterns of exploratory behaviour. None of the other interactions was significant (see appendix for details).

c) Numbers of Squares Entered:

Analysis of variance over the six test mazes and eight trials per day for the total squares entered measure, typically considered a measure of activity, again failed to find any significant differences between the groups $F(2,36)=0.12$ $p>0.05$. Furthermore, as before, no significant trials by experimental groups effects emerged $F(14,252)=0.70$ $p>0.05$, although the expected trials effect was present $F(7,252)=20.31$ $p<0.001$. As can be seen from Figure 7:5, which describes the number of squares entered by the three experimental groups over the eight trials (collapsed across the six test mazes) offspring scores decreased over trials, presumably as they quickly learned their way through the mazes. As before, a significant maze effect emerged $F(5,180)=11.83$ $p<0.001$, in that some mazes inspired more activity than others (mean number of squares entered per maze, collapsed over trials for the three groups: Maze 1: 20.49; Maze 3: 28.11; Maze 5: 27.79; Maze 7: 32.76; Maze 9: 28.12 and Maze 11: 18.79). However, in this case a significant maze by group interaction did emerge $F(10,180)=6.16$ $p<0.001$, suggesting differential performance on the six mazes by the three offspring groups. As can be seen from Figure 7:6, which graphically represents this interaction, SEC offspring were more active in the later mazes whilst apart the fifth maze (maze 9) IC offspring were less active over days. Finally, although there was no trials by maze interaction $F(35,1260)=1.34$ $p>0.05$, there was a significant trials by maze by groups

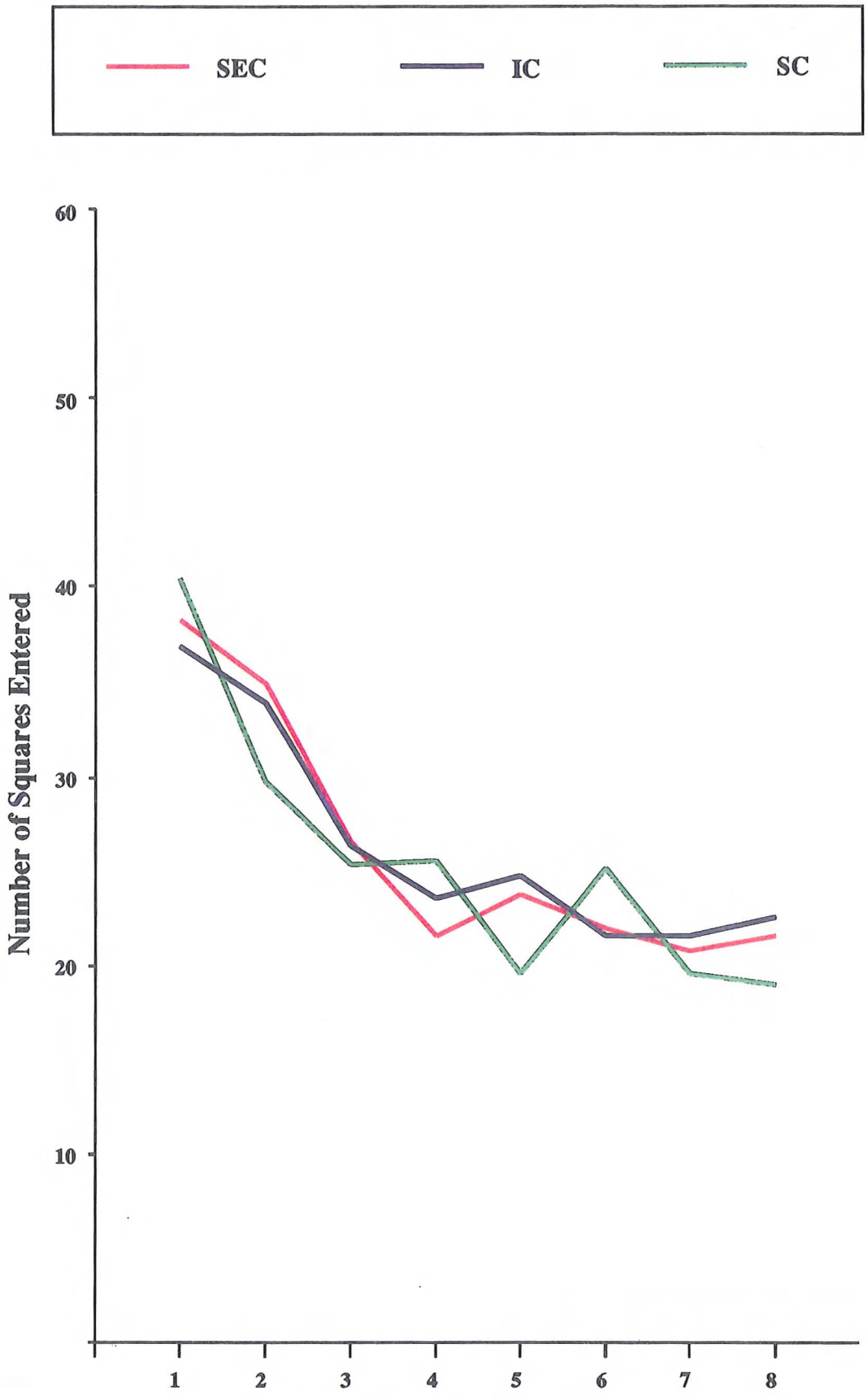


Figure 7:5

Mean number of squares entered by the three offspring groups over eight trials, scores collapsed over the six test mazes.

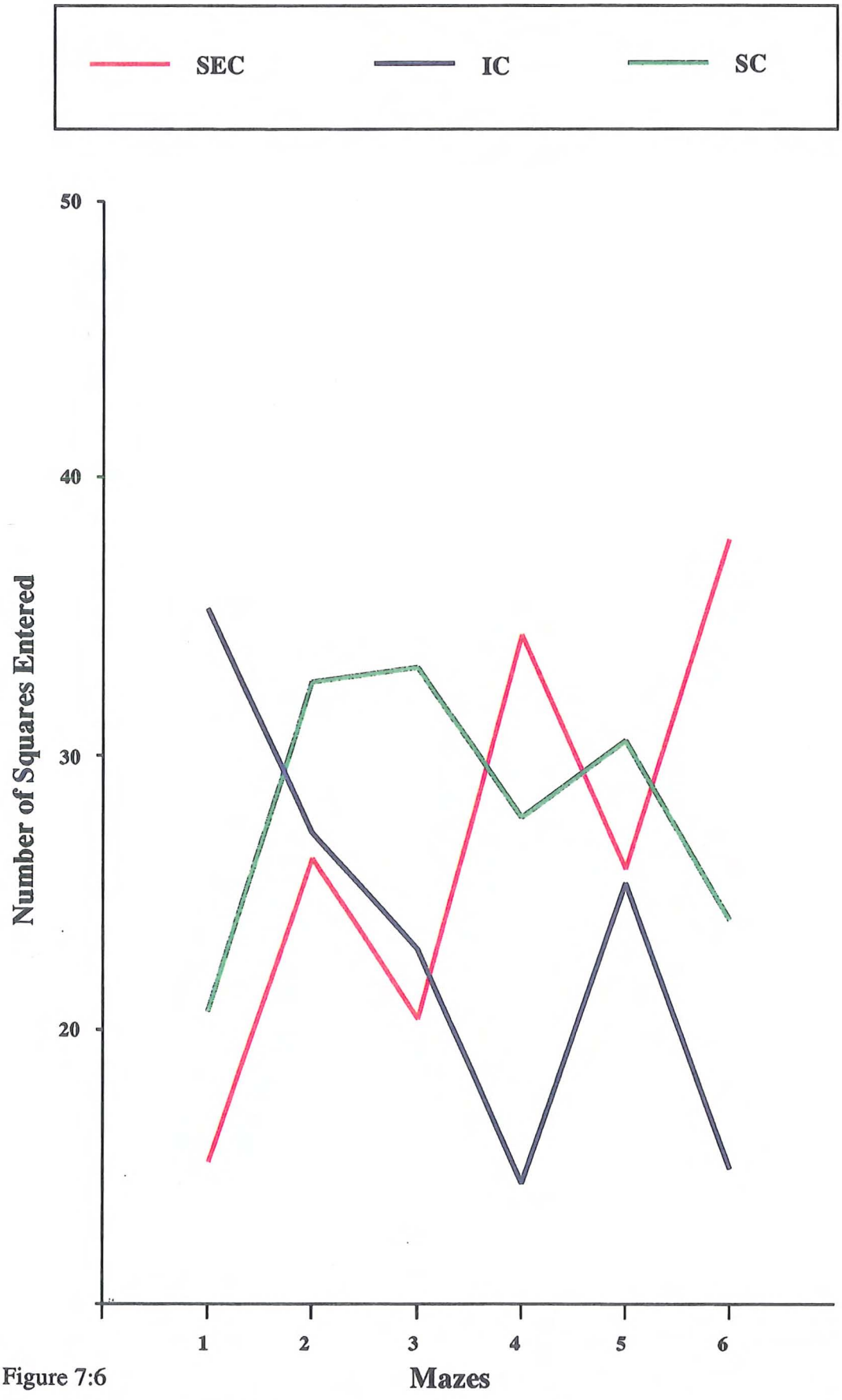


Figure 7:6

Mean number of squares entered for the three offspring groups over the six test mazes, scores collapsed over the eight trials per maze.

interaction F(70,1260)=1.36 p<0.02, offspring groups demonstrating different patterns of responding over the various mazes (for means of the groups see the appendix).

d) Latency to Leave the Start Box:

As the only explicit measure of emotionality in the Hebb-Williams battery of dependant variables, an ANOVA of latency to emerge into the maze was carried out on the animals' scores over mazes and trials. As with the previous variables no significant groups effects emerged F(2,36)=1.76 p>0.05 but there was the expected trials effect F(7,252)=17.31 p<0.001, animals taking longer to leave the box on trial one than on any other trial. Moreover, a highly significant group by trials effect was also found F(14,252)=3.81 p<0.001, which was due to the IC offspring taking considerably more time to emerge from the start box than SC or SEC offspring, on the first trial [12]. This can be seen from Table 7:4, which gives the means of the three groups over the eight trials, collapsed over the six days of testing (the six mazes).

GROUP	TRIAL 1	TRIAL 2	TRIAL 3	TRIAL 4
SEC	6.29	0.82	1.49	1.58
SC	16.91	2.19	1.01	0.71
IC	34.35	4.44	1.40	0.88
GROUP	TRIAL 5	TRIAL 6	TRIAL 7	TRIAL 8
SEC	2.79	3.54	0.59	0.86
SC	0.51	1.08	0.46	0.78
IC	0.68	0.42	0.51	0.71

Table 7:4 Means of the latency to leaves the start box of the three offspring groups over the eight trials, collapsed over the six test mazes. SEC=offspring of the SEC dams, SC, the offspring of SC dams and IC, offspring of IC dams.

In this measure, no other significant effects emerged. For further details please see the appendix.

[12] Interestingly, SEC offspring were slower than SC and IC progeny on the later trials although this latter result was less pronounced.

e) Time to Reach Goal Box and Total Time:

Considering the last two dependant variables together, no significant differences were found between the groups, with respect to either the time taken to reach the goal box (for the first time), or total time until animals had eaten all the pellets [13] $F(2,36)=2.68$ and 1.55 $p>0.05$. Significant effects did emerge over trials $F(7,252)=34.05$ and 31.01 $p<0.001$, for the two measures respectively, all animals taking less time on each consecutive trial, qualified by significant trials by experimental group interactions $F(14,252)=3.58$ and 2.36 $p<0.001$, IC animals taking longer than their SEC and SC counterparts in the earlier trials. These latter results are not unusual given the latency to leave the start box findings. Furthermore, for both the time to reach the goal box and total time measures, significant trials by maze interactions emerged, $F(35,1260)=3.14$ and 2.58 $p<=0.001$ respectively. The time to reach the goal box interaction was qualified by a groups effect too, $F(70,1260)=1.46$ $p<0.009$, typically IC and SC offspring groups taking longer on initial trials than their SEC counterparts, on the earlier mazes (see appendix for relevant means).

f) Summary of Significant Group Effects:

MEASURE	EFFECT	PROBABILITY
TRAINING		
Latency to Eat	Groups	$p<0.05$
Days to Reach Criterion	Groups	$p<0.08$
Day One Time to Goal Box	Groups	$p<0.007$
TESTING		
Squares Entered	Maze by Group	$p<0.001$
Latency to Emerge	Trials by Group	$p<0.001$
Time to Reach Goal Box	Trials by Group	$p<0.001$
Total Time	Trials by Group	$p<0.001$
Time to Reach Goal Box	Group by Maze by Trials	$p<0.009$

Table 7:5 Summary table of all significant effects involving the three offspring groups, in both the training and testing phases of the Hebb-Williams maze.

[13] This measure of total time includes time to reach the goal box, any retrace time and time to eat all the pellets in the goal box.

As can be seen from Table 7:5, which details the significant findings in this study, most of the differences between the groups emerged in the dependant variables measuring activity and emotionality. In particular in the pre-training phase, SC progeny took longer to eat in the goal box than their SEC counterparts whilst in the training maze (day one) IC and SC progeny took longer to reach the goal box than the SEC progeny. The other main effect during the training phase, days to reach criterion, was only significant at 8% and must therefore be treated with caution. As well as the group main effects, several group interactions also emerged, all of which emerged in the testing phase of the procedure. Typically, IC animals took longer in the initial trials in latency to emerge, time to reach the goal box and total time taken in the six test mazes than their SC and SEC counterparts suggesting these animals were more emotional, whilst patterns of activity in the three groups differed over the six mazes, IC progeny being more active in the early mazes, SEC in the later ones.

7:2:4 DISCUSSION

The results of the present experiment have demonstrated that, unlike offspring of mothers exposed to differential environments *during* pregnancy (Kiyono et al 1982; 1985), or offspring experiencing both prenatal and preweaning enrichment (Denenberg et al 1968), offspring of mothers raised in SEC, SC and IC *prior* to pregnancy do not differ from each other in terms of their problem solving ability, as measured in the Hebb-Williams maze. However, as can be seen from Table 7:5, some significant differences did emerge between the offspring groups, in interactions in variables associated with activity and emotionality, which warrant further discussion. Although clearly speculative, these latter results suggest that any differences between the groups might best be seen in the context of mediating factors such as differential stress or arousal levels rather than different cognitive abilities per se [14]. Indeed, the present results can be seen as beginning to unravel the causal mechanisms underlying the differences in offspring performance observed in

[14] This does not preclude alternative causes such as changes at neurochemical or endocrinological levels and it would be surprising if such changes were not involved. However, as no assays of any biochemicals were taken, this level of analysis is left for future research.

the previous study (chapter six), a fact which will become more obvious as the findings from this study are discussed in some detail in the next sections.

Offspring Learning Ability

In this experiment offspring learning ability was measured in two separate ways; in the training period as number of days to reach criterion and in the testing phase, as number of errors made in the six test mazes.

Considering the training phase first, there was a tendency for the SEC offspring to reach criterion several days before the SC offspring group, which in turn took fewer days to reach criterion than their IC counterparts. Although this result was only significant at the 8% probability level and must therefore be treated with caution, initially it does appear to suggest differences in cognitive capacity between the three groups. If however, the requirements for reaching criterion are examined more closely, this impression soon becomes illusory. To achieve criterion, animals were required to traverse the practice mazes over the nine trials in less than one minute, on two consecutive days. If any animal took longer than a minute then it was not deemed to have achieved criterion. Consequently factors such as time spent in the maze and latency to leave the start box contributed to the success of some of the animals in the early days of training. Although analyses were not performed on all the days of training (as group sizes varied over days as increasing numbers of animals did achieve criterion) day one of the practice maze training phase was examined.

As can be seen from the results of this analysis, a significant difference emerged between the groups with respect to their latency to reach the goal box in this first practice maze. Inspection of the means (Figure 7:2) shows that SC and IC offspring took longer to arrive at the goal box than their SEC counterparts. Although SC and IC offspring groups' response times were longer than their SEC counterparts, it is difficult to interpret this as a genuine difference in learning ability, since there was no corresponding differences between the three offspring groups in the

second learning measure taken, error scores in the testing phase of the procedure. Furthermore, no significant differences emerged between the groups' number of errors over trials either, as would be expected if it is this measure which best illustrates differences in learning between the groups (Dell and Rose 1986) This suggests that differences in latency to reach the goal box in the training phase are more likely to be due to emotionality and/or activity differences than differences in cognitive ability.

This lack of significant differences in learning between the groups is particularly interesting given that Kiyono and his colleagues (1982; 1985) have found beneficial effects of enrichment in offspring of animals exposed to differential environments during pregnancy. One obvious difference between the methodology employed in the present work and that of the Japanese researchers is the *timing* of the environmental manipulation. As noted before, it may well be, for example, that exposing pregnant females to enrichment has a "direct" impact on their offspring in utero which alters their cognitive capacity, an effect that could not occur in the present paradigm, all pregnant animals being housed in similar environments, namely parturition cages [15]. Indeed, Kiyono et al (1985) have suggested that this may be occuring, when they state that "fetal life is closely related to the outer world, not only with the intrauterine environment but also with the external world via the maternal body" (p434). They do, however, note that the mechanism by which this "prenatal maternal enrichment has a beneficial effect on postnatal learning of the offspring... remains to be solved" (p434).

The timing of the maternal manipulation, however, is not the only factor that should be taken into account, when trying to understand the present results. Several other elements may have contributed to the lack of significant differences between the groups. Firstly, as noted in the results section, in this experiment very few animals having reached the goal box, went back into the maze to re-trace their paths. Re-tracing errors have been found to contribute to the performance differences in animals exposed directly to enriched and impoverished environment

[15] An alternative suggestion, is that their measure of cognitive capacity was confounded by an activity or emotionality measure. However the details given in their study do not allow this speculation to be explored further.

(Woods 1959) with IC animals indulging in more re-tracing than their EC counterparts, thus increasing the number of errors they scored. It may well be therefore, that the lack of re-tracing observed in this experiment contributed to the similarity in the performances of the SEC, SC and IC offspring [16].

Secondly, the animals employed in this experiment were comparatively young, training starting at 22 days of age. At this time in their lives, rats are still growing rapidly and eating to maintain growth (see growth chart in chapter four). As the animals in this study were being rewarded for reaching the goal box with food, it may well be that inadvertently the methodology established a strong drive in all the animals to reach the goal box as quickly as possible, thus reducing any tendency for offspring to linger in the maze and "collect" error scores. Indeed, no significant differences were found between the groups in the time it took them to reach the goal box in the testing phase, as would be predicted by this explanation. Furthermore, the idea that animals that are highly motivated to reach the goal box produce comparable error scores has already been demonstrated in the EC/IC literature. Woods et al (1961) have reported, for example, that under high levels of food deprivation restricted animals' performances were equivalent to that of their EC counterparts.

However, the fact that animals in the present study were young when they were trained and tested and perhaps highly motivated by food deprivation is not sufficient to explain the present lack of differences between the groups. Kiyono et al (1985) for example, have demonstrated significant differences between offspring of animals maintained in enriched and impoverished environments whilst pregnant, when training and testing in the Hebb-Williams maze had been started at weaning, a procedure comparable to the present one [17]. It is unlikely, therefore that age of testing and high levels of motivation can explain the present findings adequately.

What does appear to be clear, however, is that the effect of exposing females to differential

[16] It should also be mentioned here that re-tracing can also be seen as a measure of activity. No differences between the groups in this experiment could therefore be seen as evidence of similar levels of activity.

[17] It should however be noted that Kiyono and his colleagues employed water as a reward in the maze, rather than food, although this is also likely to have set up a strong drive in the animals.

environments prior to pregnancy produces offspring that are qualitatively different from those of animals exposed to environments during pregnancy.

So what can be deduced about the nature of the offspring differences, and mediating mechanisms thus far? Firstly, it now appears unlikely that exposure to mothers with different experiential profiles produces offspring with different cognitive capacities. This suggests that the differences observed in offspring Skinner box behaviour reported in the previous study did not reflect any differential cognitive abilities and that some other factors must be causing the effect. Secondly, in this study, the offspring criterion differences were more easily explained in terms of emotionality than learning, opening up the possibility of a role for stress and/or arousal in mediating the offspring effects. To further explore this idea, offspring activity, exploration and emotionality measures will be discussed and their contribution to the offspring profiles highlighted.

Offspring Activity, Exploration and Emotional Responsiveness

Within the overall profile of data outlined in the results section there are clear indications of differences in levels of activity, exploration and emotionality in the Hebb-Williams test situation.

For example, although there were no overall differences between the offspring groups in the squares entered measure in either training or testing phases, there was a significant group difference in terms of patterns of activity over the six mazes used in the testing phase. This suggests that any explanation of offspring differences must encompass differential interaction with the characteristics of the test situation. This viewpoint is further reinforced by the finding that there was a significant difference between the number of rearing responses (argued to be a measure of exploration by Dell and Rose 1986) made by all the groups in the six test mazes. In measures of emotional responsiveness too, differences between the groups were also noted. Three separate measures of emotionality were taken, latency to eat all the Noyes pellets in the goal box, latency to enter the runway in the first phase of training and latency to leave the start box during the testing phase of the Hebb-Williams procedure. Considering the results of the initial

phase of training first, offspring of SC animals took longer to eat their pellets in the goal box than their SEC counterparts. Why the SC progeny were more influenced by being placed in a novel environment than the SEC progeny is not clear. What is clear, however, is that by the second phase of training, in the practice mazes, both the SC and IC animals were taking longer to complete the maze, whilst in the testing phase of the procedure, although no significant group main effects were found, when patterns of behaviour were examined over trials, offspring of IC dams took longer to emerge in the first few trials irrespective of maze, than either their SEC or SC counterparts, suggesting that even after an extensive training phase, these animals were the most emotional.

When looking at the whole picture, however, the data are complex and do not lend themselves to complete explanation in terms of either activity, emotionality or exploration. For example, if offspring differences were simply reflecting differences in activity, then a consistent pattern of differences in both the training and testing phases would have been predicted. Although differences were found between the groups' activity patterns over days in the testing phase of the procedure, no significant differences emerged between the three offspring groups with respect to number of squares entered in the first training maze (maze A), nor were there any differences in patterns of responding over trials between the three groups in this maze, although all animals did reduce their activity over trials, as would be expected. Similarly, no differences between the groups were observed in exploration. Furthermore, when considering measures of emotionality, although differences did emerge between the groups in all three measures taken, the relative pattern of differences between the groups changed as the Hebb-Williams procedure progressed.

In reality, it is likely that both activity and emotionality are involved, perhaps interactively. In these circumstances the most likely way of teasing out the relative contribution of these factors and the relative roles of underlying processes such as arousal and/or stress is to attempt direct manipulation of one of the hypothesised mediating variables and to observe the effects. Consequently, in keeping with this argument, it was decided to attempt to manipulate arousal.

The choice of arousal over stress at this juncture reflects the fact that specific predictions about directions of effects can be made more easily, by invoking relative positions of the offspring groups on an inverted-U arousal-performance curve (Yerkes and Dodson 1908). Stress-performance effects, as has become clear from chapter three, are less easily prescribed, relying as they do on the nature of the stressor and the task employed.

Summary and Conclusion

The main purpose of this experiment was to further analyse the nature of the offspring differences observed in experiment one of the previous chapter. Reasoning that if the offspring differences that emerged in those data did reflect varying learning capacities, perhaps engendered by the different learning opportunities afforded the offspring by their qualitatively different mothers, then these cognitive differences should be most obvious in tasks which directly test for learning ability. Consequently, offspring performance in the Hebb-Williams maze was tested. In addition, it was thought that because of the nature of the procedure employed in this experiment, offspring activity, exploration and emotionality could also be monitored, further elucidating offspring patterns of behaviour. Both of these aims have been achieved.

The most important finding of this study was that whatever the nature of the offspring differences, they are not due to differential learning capacities per se. Specifically, it was predicted that if SEC mothers were altering the cognitive abilities of their offspring in some undefined manner, then SEC pups should make less errors in the Hebb-Williams maze(s). Furthermore, their learning curves over trials should distinguish their performances from those of the other offspring groups. Neither of these hypotheses was supported by the data. Instead, the behavioural pattern that has emerged is one of differences in activity and emotionality between the three groups.

Why these three progeny groups are behaviourally different, however, is less clear. One explanation that has been advanced earlier in this thesis, was that the offspring groups were differentially aroused. If this were the case, then certain predictions could be made about their performance.

It is now "well known that too much or too little arousal is deleterious to a number of central nervous system functions ranging from cellular metabolism to behavioural performance" (Walsh and Cummins 1975 p990). Typically, it is the behavioural aspects of optimal levels of arousal that have been most researched and it has been suggested by some (Yerkes and Dodson 1908; Hebb 1955; Malmo 1959; Schlosberg 1954) that the relationship between arousal level and behavioural efficiency can best be described as an inverted U-shaped curve, that is high and low levels of arousal producing poor performance, maximum performance only being acheived with an optimal level of arousal, typically around the mid-point of the continuum. These "optimal levels of arousal" can vary between individuals and from one task to another (Duffy 1957; Lacey 1967; Schlosberg 1954) but the basic principle remains the same. In a second respect, also, arousal level is intimately linked with performance, to the extent that organisms will deliberately seek to maximise or minimise their levels of arousal to obtain the optimal level for the task in hand. One technique to manipulate levels of arousal available to subjects is to deliberately seek or avoid stimulation (Berlyne 1960; Hebb and Thompson 1954; Leuba 1955; Ludwig and Stark 1973; Schneirla 1959); that is behaviourally alter or control stimulus levels and thus arousal levels.

When considering the three offspring groups' performances, there is some evidence that their behaviour can be seen in the context of differing baseline arousal levels and subsequent alterations in stimulus seeking behaviour. For example, when examining the SEC offspring performances in more detail, these animals were less emotional on all training and testing phases than either the SC or IC offspring groups. That is, these animals were less reactive, consistent with the idea that they had lower arousal levels than either their SC or IC counterparts. Furthermore, day one of testing produced SEC progeny that were less active than the other two offspring groups, again consistent with the idea of lower baseline arousal levels. These animals also increased their activity over days relative to their SC and IC counterparts. This latter finding might tentatively suggest that the SEC progeny are stimulus seeking in the later mazes, to optimise their arousal levels, as they had quickly habituated to the novelty of the test environment. If this were the

entire story, however, these animals would also have increased their rearing behaviour over days, when compared with the SC and IC offspring groups. This was not the case.

Considering next the offspring of IC animals, these animals only started to habituate to the mazes as the test days progressed, reflected in their maintaining higher levels of emotionality throughout the test period and only reducing their activity on the last few mazes. This is not inconsistent with an arousal hypothesis. If these animals were initially more aroused, they would presumably take longer to habituate to the test environment and produce higher levels of emotionality and activity in the initial trials, as was found. Similarily, the SC progeny's activity profile over days is not inconsistent with an arousal hypothesis. On a note of caution, however, if all that distinguised the three offspring groups' performances were differing baseline arousal levels, then consistent differences across all test situations should have emerged. As noted in the previous chapter, this was not the case. It may be that arousal differences are contributing in part to the offspring groups' behaviour, but clearly this is not the only explanation.

Indeed, there is a large literature which demonstrates that prenatal stress can also cause behavioural effects in the offspring. Furthermore, these effects are more likely to occur in measures of activity and emotionality (Daly 1973; Denenberg 1964; Denenberg and Haltmeyer 1967; Levine 1969) reminiscent of the present findings. It may be therefore that the offspring are being differentially stressed by their encounters with their mothers, the effects being mediated by changes in their neuroendocrine systems (see chapter three). Of course this idea relies on the possibility that enriched, standard and impoverished conditions are differentially stressful, an hypothesis which is still open to some debate (Uphouse 1980; Renner and Rosenzweig 1987). Despite this, however, the data do seem to offer partial support for the idea that offspring groups are differentially stressed.

For example, it has been found that stimulation of the foetus applied through the mother can influence the emotional behaviour of her offspring (Thompson 1957a; Ader and Belfer 1962b; Thompson and Quinby 1964; Morra 1965b), typically offspring of stressed animals being found

to be more emotional than offspring of non-stressed controls. Indeed, when cataloguing the effects of prenatal stress on offspring, certain similarities start to appear between offspring of stressed dams and the IC progeny. For example, Thompson (1957a) has reported that prenatally stressed animals demonstrate longer latencies than controls in an emergence test, as do IC offspring in this experiment. Futhermore, in a maze learning test (Lamp 1967), prenatally stressed offspring did not differ from control offspring, no differences emerging between SEC, SC and IC offspring in this study either. So, there is some evidence that the offspring effects are similar to those caused by prenatal stress.

However, not all of the data are consistent with the idea that the offspring groups are differentially stressed. For example, in this study the "control" group (offspring of the SC housed animals) were as slow to complete the practice maze for food as their IC counterparts, both groups differing from the offspring of SEC dams. This pattern of behaviour is inconsistent with prenatal stress predictions. Furthermore, although prenatally stressed animals have been found to be less active than controls (Denenberg and Whimbey 1963; 1968; Denenberg and Rosenberg 1968), reminiscent of the IC progeny's performance over days in the test maze relative to their SC counterparts, these performances have not been found to be consistent across behavioural tests (cf open field results, chapter six). This does not further the case for prenatal stress being *the* cause of the offspring differences.

To reiterate, therefore, it is unlikely that the offspring profiles observed to date reflect differential learning capacities per se, but are most likely to be the product of activity and emotional changes. Although mediating mechanisms still remain elusive, it now seems unlikely that differential opportunities for learning afforded the offspring by their mother postpartum can explain the results. It is more likely that either the effects of maternal stress mediated in utero, or alterations in offspring arousal by maternal stimulation postpartum may be causing the offspring differences. Obviously these are not mutually exclusive mediating factors. Indeed, given the lack of consistent findings across various test situations, it may be that both stress and arousal (and

their accompanying changes in biochemistry at neuronal and endocrine levels) are interacting to produce the observed effects. However, suggesting that offspring groups are differentially stressed and/or aroused proffers no more information than describing their behaviour in terms of emotionality and activity differences. What is required is a test of one or other of these underlying mechanisms by directly manipulating them and observing the resultant changes in offspring behaviour. This provides the focus for the next experiment, in which offspring *arousal* levels were artificially manipulated in a Skinner box procedure devised by Rose et al (1986).

7:3 EXPERIMENT TWO

7:3:1 INTRODUCTION

In 1975 Walsh and Cummins proposed that a 'fundamental mechanism" in understanding "the anatomical and biochemical brain changes induced by exposing animals to environments rich in sensory stimuli... is the arousal response, since alterations in arousal appear to be concomitant with all such environmentally induced changes" (p986). In particular, they argued that EC animals, being subjected to higher stimulation in their environments than their IC counterparts are subsequently less likely to exhibit arousal reactions when placed in a variety of tests. The concept of differential baseline arousal levels has also been proposed as one explanation of the behavioural differences found in offspring of animals exposed to SEC, SC and IC prior to pregnancy observed in the present thesis. In this experiment, the hypothesis that offspring performances can be explained in this way is further explored.

In particular, the experimental evidence gathered so far has provided a behavioural profile which although not consistent across test situations, suggests that the arousal homeostasis of the offspring groups may differ. Given that arousal levels are intimately linked with performance such that organisms are motivated to behaviourally manipulate their arousal levels by seeking or avoiding stimulation (Chadha and Rose 1981) in order to obtain an optimal level for any particular

task (Berlyne 1960; Hebb and Thompson 1954; Leuba 1955; Ludwig and Stark 1973; Schneirla 1959), it follows that by externally raising stimulation levels, subjects' behaviours will also be changed.

Within the EC/IC literature, one such procedure has already been developed (Rose et al 1986; 1987) in which high levels of stimulation are used as a reinforcer in a Skinner box task. In particular, Rose et al (1986) employed a complex reinforcer to be used in conjunction with an adapted Skinner box, comprising tactile stimulation from pressing the Skinner box lever paired with auditory stimulation from the lever microswitch, further auditory stimulation was provided by the sound of the pellet dispenser operating, whilst visual stimulation consisted of one second of varying intensities of illumination [18] and a pellet of food. They argued that the behaviour of animals exposed directly to EC and IC may be differentially reinforced by the same response-contingent events and developed their technique to equate "reinforcers for EC and IC subjects" in order to more clearly evaluate the effects of environmental enrichment on learning divorced from effects mediated by differential motivation in these subjects.

In the present thesis, of course, the concern is with offspring of differentially reared animals. If these animals do differ in terms of basal arousal, then certain predictions about their Skinner box behaviour can be made. In the previous Skinner box study, SC offspring were found to bar press significantly more than their SEC counterparts, IC animals, although not differing statistically from either the SEC or SC groups, producing bar press levels which fell in between their SC and SEC conspecifics. These performances might be seen as reflecting different positions on the sort of inverted-U arousal-performance curve described earlier, such that SC animals being optimally aroused produced optimal performance levels, whilst the lower performances of the SEC and IC progeny reflected their less than optimal arousal levels. Whether SEC and IC offspring had arousal levels which were lower or higher than their SC counterparts, is as yet unclear, as both could produce similar levels of performance. What is relevant to this introduction, however,

[18] The reinforcement illumination levels were set at either dim (53 foot lamberts), medium (750 foot lamberts) or bright (2,500 foot lamberts) settings.

is that by employing a composite reinforcer which is highly stimulating and which would be expected to result in the raising of all offspring groups' arousal levels, a shift of all three groups along the hypothesised arousal-performance curve would be predicted, such that the rank ordered position of the offspring groups, in terms of bar press rates, would change. Specifically it would be predicted that SC progeny performances would drop and SEC and IC offspring either raise or drop their performance levels depending on their initial position on the curve.

Finally, in this experiment, as with the previous one, only male animals were employed. This decision was based on practical constraints as only a limited number of modified Skinner boxes were available and to include a group of female offspring would have made the procedure on each day far too long to carry out. Obviously, animals could have been bred in two batches, as in the previous experiment, but additional constraints were imposed in terms of colony room space at this time.

7:3:2 METHODOLOGY

a) Subjects:

These were 57 male F2 generation Hooded Lister rats of weanling age (21 days), 19 animals being bred from F1 generation females exposed to either SEC, IC or SC prior to pregnancy, following the procedure outlined in the general methodology chapter (chapter four). Group sizes reflected the maximum number of available males born to the SEC, IC and SC dams and testing constraints outlined earlier.

b) Apparatus:

Based on the modifications developed by Rose, Love and Dell (1986), three Skinner boxes of the Type I variety described in the general methodology chapter were employed in this experiment.

c) Procedure:

At weaning, subjects were weighed and assigned to individual cages for a day prior to the start of testing. This was to allow the animals to settle following separation from their mothers and siblings and to place them on a maintenance diet of eight grams of breeding diet at the end of each day. Starting at 22 days of age, subjects were given an 18 day Skinner box procedure based on that employed by Rose et al (1986). Specifically, training consisted of daily 30 minute sessions, according to the following sequence of schedules: CRF (Days 1-5); FR3 (Days 8-9); FR6 (Days 10-12 and 15-18). No training was carried out on Days 6, 7, 13 and 14, subjects being fed ad libitum on these days. Throughout the experimental procedure animals were weighed daily to ensure normal growth rates were maintained. Subjects were run in groups of three and animal running order was randomised on each day of testing.

Reinforcement in this experiment consisted of the sound of the lever microswitch paired with the sound of the pellet dispenser, supplemented by one second of illumination of the white perspex roof of the operant chamber and a pellet of food. In this instance, the light was set at 750 foot lamberts, equivalent to Rose et al's (1986) "medium" setting. The decision to use this light setting was based on several factors. Firstly, a low light level had already been employed in the previous Skinner box experiment, so in parametric terms, the obvious choice would be a medium light level to increase stimulation. Secondly, in Rose et al's experiments (1986; 1987) the medium setting was the lowest light level which clearly separated out the EC and IC animals (53 foot lamberts producing similar behavioural profiles in these animals) whilst providing high levels of stimulation. Finally, although the brightest light (2,500 foot lamberts) might also have been included, experience with this latter light level has demonstrated that it generates a degree of heat in the Skinner box which might be aversive rather than reinforcing. After each trial number of bar presses were recorded, equipment cleaned and relay counters re-set for the next group of animals.

7:3:3 RESULTS

Unlike the previous experiments, in the present work analysis of the littersize effects, checked using a one way ANOVA revealed significant differences between the three experimental groups $F(2,54)=12.76$ $p<0.001$. As can be seen from Table 7:6, which describes the means of the groups, SC offsprings' average littersize was larger than either IC or SEC offspring groups'.

GROUP	LITTERSIZE
SEC	9.16
IC	10.53
SC	12.79

Table 7:6 Mean littersize of the three offspring groups.

Consequently, in the present experiment littersize was included in the analysis of number of bar presses, as a co-variate. Furthermore, as explained in the general methodology chapter, the use of co-variance requires that there is homogeneity of variance between the groups (Harrington 1968). This was tested using the Bartlett-Box F test.

a) Skinner Box:

Prior to analysing the data, homogeneity of variance between the three offspring groups was calculated on subjects' scores totalled over the fourteen days of testing. As no differences emerged, Bartlett-Box $F(2,6561)=2.05358$ $p>0.05$, parametric statistics were employed. As can be seen from Figure 7:7, which shows the learning curves of the three offspring groups, SEC and SC animals bar pressed significantly more than their IC counterparts. This was confirmed by an analysis of co-variance of the three groups' bar press scores over the fourteen days of testing. Highly significant group effects emerged $F(2,53)=10.92$ $p<0.001$, qualified not unexpectedly given the learning curves of the three groups, by a significant offspring group by days interaction $F(26,702)=4.68$ $p<0.001$.

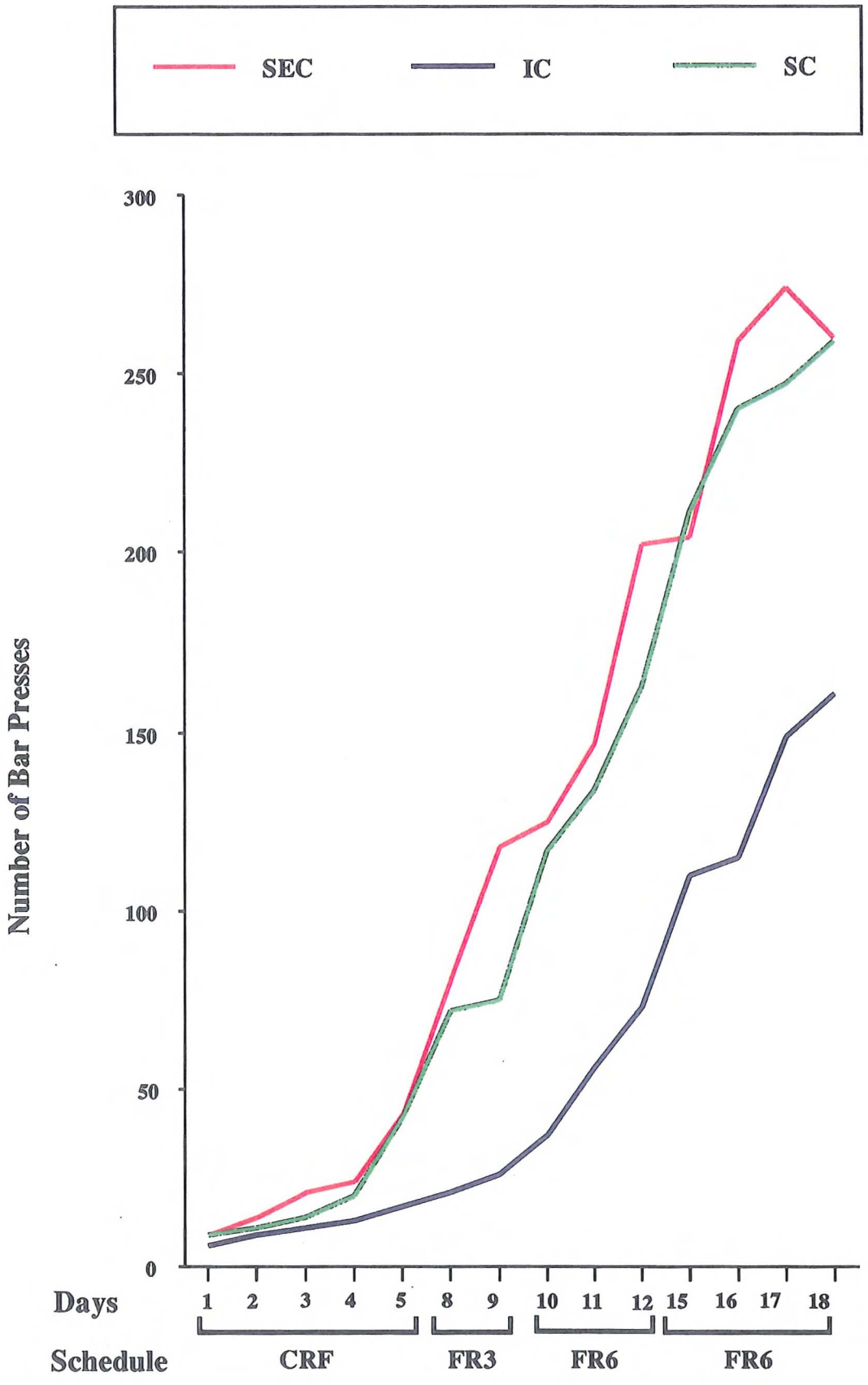


Figure 7:7

Mean number of bar presses for the three offspring groups over the eighteen days of Skinner box training, in the Type I boxes.

Finally, a highly significant days effect also emerged $F(13,702)=118.52$ $p<0.001$.

7:3:4 DISCUSSION

Unusually, in this experiment offspring littersizes varied between the three groups, with SEC animals producing smaller litters than their IC and SC counterparts. There is no obvious reason for this, all procedures employed in this experiment being consistent with those of previous experiments and is most likely a chance occurrence. However, as prescribed in the general methodology chapter, littersize, which has been found to affect offspring activity (Seitz 1954), was taken into account in the analyses.

The results of the present experiment demonstrate significant differences between the offspring groups and, in part, support the experimental hypothesis. However, once again, the overall picture is one of considerable complexity. In the introduction it was suggested that the Skinner box performances of the three offspring groups in chapter six, in which to recap, offspring of SC animals bar pressed more than offspring of SEC and IC mothers [19] might reflect their differing levels of arousal. In particular, it was hypothesised that SC offspring might be optimally aroused for that task and produced an efficient performance, seen as high bar press rates, whilst the SEC offspring and IC offspring were either under or overaroused, producing "poor" performances, that is similar levels of bar pressing to each other and at a lower rate than the SC group. In the present experiment, however, with the use of a composite reinforcer with a highly stimulating burst of light, SEC offspring produced equally high bar press rates as their SC counterparts, both groups differing significantly from the offspring of IC animals.

If, as has been proposed, one considers the offspring of SEC, SC and IC dams to be differentially aroused, in the present experiment the strong stimulating properties of the composite reinforcer would be particularly rewarding for some animals and would result in their producing high levels of bar pressing. This is exactly what was found in the SEC group. In the previous

[19] NB: SC offspring were statistically significantly different from SEC offspring but not from IC offspring.

experiment, in contrast, the reinforcer was not as stimulating for these animals and was thus not rewarding enough to increase their levels of performance [20]. In both experiments the lower levels of responding of the IC offspring might be seen as avoiding additional stimulation. After all, if IC offspring's bar press responses in the previous Skinner box experiment had been due to low arousal levels, then their performances would have gone up in this study. They did not, suggesting that these animals are overaroused and that the response contingent reinforcement in both experiments was too stimulating and thus was not rewarding.

So, both the SEC and IC offspring groups are behaving in a manner consistent with the hypothesis that they are differentially aroused. However, as is becoming increasingly common in this thesis, not all of the data fits this explanation. In this case, the behaviour of the SC progeny does not obviously conform to the experimental predictions. In particular, it was suggested that increasing these animals arousal levels through the stimulating properties of the reinforcer would shift them along the arousal-performance curve resulting in a reduction of their bar press rates relative to those of the SEC offspring. As can be seen from Figure 7:7, this was not the case. The performance of the SC progeny was statistically indistinguishable from that of the SEC offspring. This result could be interpreted as evidence that the SC offspring were more aroused than their SEC counterparts (as was predicted based on their bar press rates in the previous Skinner box experiment) but that the increase in their level of arousal, due to the present composite reinforcer, although shifting them along the arousal-performance curve, moved them from one point on the left hand side of the curve to a similar point on the right hand side of the curve. This would produce identical levels of performance, subserved by differing levels of arousal. However, the validity of this explanation cannot be checked and it suffers from the criticism that the data is being forced to fit an arousal hypothesis, post hoc. What is required is a definitive test of the arousal hypothesis, in which clear parameters are established. If the offspring groups' performances do not conform exactly to the predictions, then the notion that these animals are differentially aroused will have to be abandoned and other lines of inquiry pursued.

[20] In that task, the level of stimulation afforded by the reinforcer was particularly rewarding for the SC group.

One way to more fully investigate the hypothesis that the three offspring groups are differentialy aroused, is to manipulate the animals' arousal levels pharmacologically, rather than changing the stimulus situation. This provides the focus for the next study in which the arousal levels of the three offspring groups were manipulated and behaviour tested in both a Skinner box procedure (similar to the one employed in this experiment) and in the open field.

7:4 GENERAL DISCUSSION AND CONCLUSION

The purpose of the two experiments reported in the present chapter was to investigate further the nature of the differences found between the offspring groups. In particular, the experiments in this chapter focussed on three possible mechanisms mediating the behavioural profiles of the offspring described in the previous chapter, arousal, stress and learning.

In the first experiment the Hebb-Williams maze was used and no differences were found in offspring learning abilities, although clear differences did emerge between the groups with respect to activity and emotionality. It was argued that these results might reflect either differential arousal levels in the three offspring groups or varying responses to prenatal stress. What seemed clear, at this stage, was the lack of learning differences between the groups and it was suggested, therefore, that differential offspring behaviours did not result from the different learning opportunites afforded them by their qualitatively different mothers. As the notion that offspring groups were differentially aroused or prenatally stressed seemed to be emerging as more reasonable explanations of the offspring groups' performances and that of these two explanations, manipulation of arousal allowed specific predictions to be made based on an arousal-performance curve, it provided the focus for the second experiment.

The results of this second experiment, in which a composite and highly stimulating reinforcer was used to manipulate arousal levels, provided some support for the "arousal hypothesis" In this instance, SEC offspring, whose previous Skinner box performance was significantly lower than their SC counterparts increased their bar press rates to parallel those of the SC subjects

and differed significantly from IC subjects. This increase was predicted, in terms of stimulus seeking behaviour as would be expected in animals whose arousal homeostasis was low (offspring of SEC animals) and stimulus avoidance in animals whose baseline arousal levels were high (offspring of IC animals). However, although the SC progeny's performance could be seen as consistent with an arousal hypothesis, it does not correspond exactly to the specific experimental hypothesis. To place this group's behaviour in an arousal framework was considered to rely too heavily on post hoc interpretation. Consequently, before drawing any conclusions about the the nature of the offspring differences and their underlying arousal levels, a final experimental procedure was suggested to test the arousal hypothesis to its limits, namely a parametric study of offspring performances in which arousal levels are directly manipulated, using varying doses of amphetamines. This procedure provides the focus of the next and final experimental chapter of this thesis.

CHAPTER EIGHT: STUDY FOUR

A CONSIDERATION OF THE HYPOTHESIS THAT OFFSPRING OF SEC, SC AND IC DAMS ARE DIFFERENTIALLY AROUSED: MANIPULATION OF AROUSAL LEVELS WITH D-AMPHETAMINE SULPHATE

8:1 INTRODUCTION

In the preceding two chapters (chapter six and seven), offspring of animals exposed to differential environments prior to pregnancy were found to exhibit different behavioural profiles. In an attempt to understand these findings, it was suggested that the performances of the offspring of SEC, SC and IC dams might reflect, amongst other things, differing baseline arousal levels, such that under the various experimental tasks, levels of arousal for any one of the three groups might be either optimal or inappropriate for efficient performance depending on the nature of the task. When considering the data, however, one fact has consistently emerged, namely that there is a *lack* of consistency in the offspring groups' performances both within and across tasks, suggesting that to consider the groups' performances as reflecting differing basal levels of arousal is too simplistic an explanation. However, in the previous study (experiment two), some support did emerged for the idea that offspring arousal levels could be shifted along an inverted-U curve in such a way as to maintain the rank ordered positions of the groups, which warrants further investigation. It may well be that differing arousal levels are contributing to some of the observed offspring differences but as is becoming increasingly clear, this is only part of the picture. In this study, the possibility that offspring groups' are differentially aroused is explored further.

Before describing the specific experimental manipulations and subsequent behaioural predictions, however, it should be noted that arousal, although popular in the literature as an explanatory construct (Anderson 1990), is not without its problems (Neiss 1988 [1]). In particular, it is both difficult to define and proffers little more causality than stating that the groups differ in terms of motor activity.

Considering these problems in more detail, with respect to the question of definition, Walsh and Cummins (1975) have suggested that the concept of arousal cannot be considered in terms of a single definition as "the state of arousal can be estimated in so many different ways" (p989). According to these authors, quite adequate physiological definitions can include "altered auto-

[1] See also Duffy 1957; Lacey 1967 as well as Hinde 1970; Miezejeski, Lamon, Collier and Hamilton 1976 cited in Einon and Sahakian (1979).

nomic or hormonal states; neurological definitions can be based on measures of the non-specific activation of the reticular formation, or generalised electrocortical activity; whilst behavioural definitions include the aforementioned altered levels of motor activity as well as observations of the orienting response". As can be seen from this summary, Walsh and Cummins have not limited the definition of arousal, but they have also suggested that "no matter how it is defined, it will involve a transitory state of generalised neurological excitation" (p989). It is in no stronger form than this that the term arousal is used in the present thesis.

A second problem with the concept of arousal concerns its relationship to motor activity. Duffy (1957) has pointed out that "confusion frequently arises between the degree of internal arousal (referred to by the concept) and the vigour and extent of overt responses" (p266). For her, the overt responses, for example motor activity, are separate from but highly correlated with arousal. However, as mentioned earlier, one problem that remains, which has been elucidated by Einon and Sahakian (1979), is the "obvious circularity in attributing differences in the level of activity to differences in the level of a single variable such as arousal" (p299). Indeed, they suggest that "if the term arousal is to be at all useful, we need to show that manipulations posited to affect arousal should affect behaviour in the same way" (p299). This latter point was taken up in the present chapter, where levels of arousal were manipulated with different doses of the psychomotor stimulant *d*-amphetamine sulphate, a decision based on the recommendations of Walsh and Cummins (1975) who have advocated its use as an "arousal-inducing agent" (p994).

Amphetamine is generally classified as a C.N.S. (Central Nervous System), psychic, psychomotor or behavioural stimulant and at low doses evokes an alerting, arousal or behavioural-activating response not unlike the normal reaction to an emergency or stress (Julien 1987). This reaction is not surprising given amphetamine's structure, which closely resembles that of norepinephrine (NE) a neurotransmitter involved in the behavioural activation associated with the fight/flight/fright response. More specifically, amphetamine appears to mimic or potentiate the action of certain brain amines (Randrup and Munkvad 1970; Robinson and Becker 1986) although it should be

noted that there is still some debate about the exact neural systems and processes that mediate its effects (Rebec and Bashore 1984).

Whatever the mediating mechanisms, in the rat, small doses of amphetamine (0.5 to 1.5 mg/kg) have been found to induce a pronounced stimulation of general motor activity (Iversen and Creese 1975), with higher doses (5 mg/kg) resulting in stereotyped motor behaviour in which isolated motor acts from the normal behavioural repertoire occur out of their normal context and with abnormal frequency. Behavioural responses to different doses of amphetamine have been observed in a wide range of procedures (Kelleher and Morse 1968; Randrup and Munkvad 1970; Grilly 1977; Pickens 1977; Cole 1977; Robbins 1981; Rebec and Bashore 1984), species (Dews and Wenger 1977) and time courses (Rebec and Bashore 1984; Evans et al 1973). Typically amphetamine affects the dynamics of behaviour, reflecting changes in interactions between the animal and its environment over time (Kelleher and Morse 1968) depending on the different doses employed. This can be seen most clearly in the open field literature (Rebec and Bashore 1984) where doses of *d*-amphatamine that range from 0.3 to 1.5 mg/kg produce "an increase in forward locomotion that is typically accompanied by mild sniffing" whilst "administration of higher doses elicits a multiphasic response that consists of early and late phases of ambulation and an intermediate phase of focused stereotypy during which locomotion is absent" (p154). Similar dose-response effects have also been found in operant conditioning paradigms (Kelleher and Morse 1968) where amphetamines tend to increase low rates of responding and decrease high rates of responding (more than one response per second) although this is modified by the dose level of the drug and schedule of reinforcement employed. For example Clark and Steele (1966) found that increasing doses of amphetamine (0.5 to 4.0 mg/kg) increased rates of responding under a fixed-interval schedule but decreased the rate of responding under a fixed-ratio schedule.

The studies mentioned so far have employed animals whose experiential backgrounds are very similar. Of particular relevance to the present thesis, however, are those few studies in which amphetamines have been employed to alter the behaviour of animals exposed to differential en-

vironments. One of the earliest studies was that of Will and Checchinato (1973) who found that *d*-amphetamine given intraperitoneally to albino rats at various doses highlighted the role of individual differences in the level of autonomic reactivity. In particular, these authors raised animals in isolation (IC) or in groups of four (SC) from weaning and trained them on a multiple schedule of reinforcement for fourteen sessions (starting at 52-62 days of age) prior to the administration of the drug. They then studied the effects of *d*-amphetamine over thirteen daily conditioning sessions, every third day rats receiving one of three doses of the drug (1, 2 and 4 mg/kg) and one control dose (saline solution), doses being injected following a randomised order. Interestingly, in this experiment, no differences in response rate were found between IC and SC animals, but significant differences did emerge between individual animals. In particular, bar pressing frequency varied as a function of the dose, either increasing or decreasing responding depending on the subject.

Although no significant differences emerged between SC and IC animals in this experiment, differential environmental experience has been found to modify the behavioural response to dopaminergic stimulating agents in the rat (Mandell et al 1973; Sahakian et al 1975; Einon and Sahakian 1979) and in the guinea-pig (Sahakian and Robbins 1975) as well as increasing the magnitude of the anatomical differences typically observed between EC and IC animals (Bennett, Rosenzweig and Wu 1973; Rosenzweig et al 1972). Furthermore, isolated and control rodents have been found to be differentially affected by drugs other than amphetamines (Balazs et al 1962; Welch and Welch 1966; 1971) with IC rats for example being less susceptible to certain CNS depressants (Friedman and Walker 1969; Einon et al 1986; Einon and Sahakian 1979) suggesting that these animals may well "develop with different responsiveness in their amine transmitter systems" (Sahakian et al 1975).

Considering the use of amphetamines in particular, Mandell et al (1973) have suggested that there is an isolation-induced sensitivity to the locomotor stimulant effects of 1.0 mg/kg *d*-amphetamine. However, as noted by Sahakian et al (1975), there are several flaws with this study including

the failure to take into account pre-drug differences in levels of activity between EC and IC housed animals. Indeed, the statistical analysis reported by Mandell et al (1973) failed to find a significant drug by rearing condition interaction, as would be predicted if the isolated rats were more sensitive to the locomotor activity effects of the drug. Furthermore, as pointed out by Sahakian et al (1975) no dose-response data nor time-course data were presented in Mandell et al's study either, nor were measures of stereotypy recorded, making it difficult to evaluate positively.

The idea that animals reared in social or isolated environments develop with differentially responsive amine systems was further explored by Sahakian et al (1975) in a behavioural analysis of these animals' responses to psychomotor stimulants. "The expectation was that isolated rats would show greater sensitivity than control rats to the psychomotor stimulating action of D-amphetamine." It was further "predicted that with increasing drug doses, stereotyped behaviour would appear earlier in the isolated animals and would remain more intense, until a ceiling level would be reached" (p196). Locomotor activity was measured in photocell cages and stereotypy ratings made based on a scale developed by Creese and Iversen (1973). Subjects were used as their own controls and were tested on two days per dose of four drugs, *d*-amphetamine (0.5, 1.5 and 5.0 mg/kg), apomorphine hydrochloride (0.1, 0.5 and 1.5 mg/kg), cocaine hydrochloride (5.0, 10.0, 15.0 and 20.0 mg/kg) and piradrol hydrochloride (3.0, 5.0 and 10.0 kg/mg). For all drug doses except 5.0 mg/kg *d*-amphetamine and all the apomorphine doses, locomotor activity was significantly increased by the drug treatment. However, there were no differences between IC and SC animals. With stereotypic behaviour, however, IC animals showed significantly more intense stereotypy than SC rats at 0.5 mg/kg, a difference which became more significant at 1.5 mg/kg but which disappeared at 5.0 mg/kg, presumable reflecting a ceiling effect. Similar patterns emerged for apomorphine, pipradrol and cocaine. These results show that response sensitivity to psychomotor stimulants can be differentially influenced by rearing experience, although in this experiment only the intensity of stereotypy was increased by isolation, locomotor activity being unaffected.

In a later study, however, activity differences were found between IC and SC animals (Einon and Sahakian 1979). In this study 0.5, 1.5 and 5.0 mg/kg of *d*-amphetamine sulphate doses were employed and drug dose, environmental background, sex, lighting conditions and timecourse of drug examined. Isolated rats were more active than their social counterparts, nocturnally tested rats more active than diurnally tested animals and female rats more active than their male counterparts. Furthermore, isolated rats reached their activity peak at lower dose levels than socially housed animals. However, Einon and Sahakian have interpreted the interaction of drug dose with diurnal cycle and sex of animal as evidence *against* a unitary arousal model, as these effects are not additive. Specifically, they argue that enhancement of activity differences caused by one factor, for example drug dose, also being produced by a second factor such as time of day or sex of animal, cannot be explained in terms of one intervening variable such as arousal, as this would be too simplistic. However, they do acknowledge that the results of their activity test data "agree broadly with the hypothesis that rearing conditions, diurnal cycle and *d*-amphetamine all act upon a central arousal process" (p302). It should be noted that in the present work the *nature* of arousal is not at issue. However, to reduce interactions between factors contributing to enhanced activity, only male animals were employed and all experimentation took place at the same time of day.

In their study, Einon and Sahakian also found evidence of rearing effects on stereotypic behaviour, isolated rats demonstrating more intense stereotypy at 0.5 mg/kg than their SC counterparts. Interestingly, in this experiment, IC animals also reacted to the injection as evidenced by their mild stereotypy following the saline dose, when compared to the SC animals. At higher doses, however, rearing differences disappeared, suggesting that the "dose dependency of the environmental modification of stereotypy may reflect variations in interactions between environmentally induced and drug-induced behaviour" (pp 303-304). In the final experiment in this study age at environmental experience was manipulated and like increased activity and slower habituation (Einon and Morgan 1976; 1978) amphetamine responses of IC animals were found to depend on isolation occuring prior to 50 days of age. In this experiment reactions to barbiturates were also

measured and found to influence isolates at any age. As the isolates' responses to CNS stimulants and CNS depressants were not similar, Einon and Sahakian have argued that "the suggestion that the isolated rat is simply hyperaroused does not withstand closer examination" (p306). This lack of hyperarousal in the IC animal does not preclude arousal differences between the groups, but does suggest that "the observed differences in activity between social and isolated rats can only be used as supporting evidence for arousal differences; it does not of itself constitute strong evidence" (p306). Alternatively, hyperarousal can be seen as just one of several differences and/or characteristics of IC animals.

Whether animals exposed to EC, SC or IC do differ in terms of arousal levels or not, is of secondary importance to the present thesis and should be seen within the context of mediating mechanisms [2] (see final discussion chapter). What is of interest, however, is whether the performances of the *offspring* of differentially housed mothers in any way reflect differing baseline arousal levels. One way to test this, is to chemically alter their baseline arousal levels and predict performance changes based on these chemical alterations. Before detailing said predictions, however, some methodological decisions made in this study need to be explained, namely choice of behavioural tests, drug doses and specific behavioural measures.

Considering the choice of behavioural test, first, in the present thesis two test procedures have demonstrated clear differences between the offspring groups, namely the open field (chapter six) and the Type I Skinner box system (chapter seven). Consequently, it was decided to employ these procedures in the present experiment. This choice was further guided by the fact that both operant conditioning paradigms (Kelleher and Morse 1968) and the open field (Rebec and Bashore 1984) have large pharmacological literatures which evaluate the use of specific measurements and procedures, which can be used to make informed choices in the present experiment.

Secondly, drug doses and administration needed to be considered. Grilly (1977) has argued that behavioural responses to amphetamines reflect an interaction and competition between schedule-

[2] As discussed in chapter six, it is suggested that the differential maternal behaviour of the three types of dams towards their offspring alters the latter's arousal homeostasis.

controlled responding and drug-induced stereotypic behaviour and/or increased motor activity. He suggests that for operant procedures with relatively low rates of responding (2-10 responses per minute) the increased motor activity with low doses of amphetamines would not necessarily compete with the bar-press response and the stimulant effects of bar-pressing can be expressed. With increasing doses of amphetamines, however, the animal spends increasing amounts of time engaged in stereotypic behaviour which competes with even relatively low rate schedule-controlled behaviour. Consequently, he recommends that only low doses of amphetamines be employed, and in particular that doses above 2mg/kg should be used with caution. Following this recommendation, the highest drug dose employed in the present study was 2 mg/kg. Interestingly, according to Grilly (1977) the effect of amphetamine on motor activity is somewhat less complicated than in operant conditioning paradigms, activity increasing dramatically above control levels with low to moderate doses of amphetamine and decreasing with relatively high doses, activity starting to decrease at 4 mg/kg and above. However, to maintain uniformity between the experimental procedures, with the open field test, as with the Skinner box procedure, only doses up to 2 mg/kg were employed.

With respect to administration of the drug, for the effects of the drug to manifest themselves at the start of any experimental procedure, at least half an hour was allowed, before any testing was done [3]. This methodology parallels the one employed by Will and Checchinato (1973) in their operant paradigm, even to the extent of giving the doses intraperitoneally and has also been recommended by Cole (1977) in his work with amphetamine and the open field.

The final methodological decisions to be made, concern the types of behaviours to be measured. With the operant procedure, the choice was fairly simple. In order to maximise responding, a procedure which employs relatively low rates of responding (less than 100-200 responses per minute; Grilly 1977) was employed, namely a simple fixed ratio schedule. With the open field, both stereotypic behaviour and activity as measured by lines crossed and number of rears could

[3] This decision was taken in consultation with Dr Martin Baxter, Head of the Behavioural Psychopharmacology Unit, Wellcome Research Laboratories, Beckenham, Kent.

have been measured. However, as there is still some confusion about the measurement of stereotypic behaviour, relying as it does on subjective interpretation of an animal's behaviour, it was decided to concentrate on the more easily quantifiable behaviours of rearing and lines crossed. Furthermore, as Randrup and Munkvad (1970) have noted that the various behavioural elements associated with stereotypy are not equally affected by amphetamine as the drug takes its course, with for example rearing and locomotion being increased before becoming suppressed at the time of maximal stereotypy (39 to 120 minutes following the drug) whilst grooming is suppressed almost immediately, it was felt that the more traditional open field measures would be more appropriate in this instance [4].

Having described the rationale underlying the methodological decisions taken in this experiment, in the final section of this introduction predictions about offspring performances under the various dose levels of *d*-amphetamine on the two behavioural test procedures, based on the arousal model will be outlined.

In particular, in the Skinner box task, it was predicted that prior to the introduction of the drug doses, SEC, SC and IC offspring patterns of bar press performance would follow those established in the previous study, that is SEC and SC animals bar pressing more than their IC counterparts and having learning curves with a steeper gradient than those of the latter offspring group. Once the drug doses were administered, however, it was predicted that the SEC and SC offspring would gradually become more aroused with each dose level and start to reduce their bar press rates such that their performances would be similar to that of the IC progeny group. More specifically, the following hypotheses can be forwarded:

- Pre-drug bar press rate over the last test days prior to drug administration will differentiate between the three offspring groups, such that SEC and SC progeny bar press more than the IC progeny.

[4] It should be noted here, that any precedents set by Einon and her colleagues to use stereotypy rather than activity apply to photocell activity monitors rather than the open field and for much longer test periods (up to two hours).

- In the saline condition, where animals are injected but are not administered any amphetamine, SEC and SC offspring will bar press more than their IC counterparts.
- As the dose levels of amphetamine increases, all groups' levels of performance will increase initially and then decrease, lowest levels of performance being found with the highest doses.
- The dose-response curves for the three offspring groups will differ, in that IC animals will reach an asymptotic level at a lower dose than their SC and SEC counterparts. This will be represented by a group by dose by days interaction.

Moving on to the open field test, it is predicted that the three offspring groups will differ in their dose-response curves. In this case, if, as has been suggested, the SEC offspring are the least aroused, then their levels of activity as measured by lines crossed and rearing behaviours will be reduced over days at a higher dose level than the other two offspring groups. Furthermore, animals only receiving saline will have behavioural profiles similar to those observed in chapter six.

8:2 METHODOLOGY

a) Subjects:

These were 90 male F2 generation Hooded Lister rats of weanling age (19-21 days), 30 being bred from each of the three F1 generation groups of females exposed to either SEC, SC or IC prior to pregnancy. Details of these breeding procedures can be found in the general methodology chapter (chapter four). Within each experimental group, subjects were allocated to one of the five amphetamine dose levels, such that each subgroup contained six animals. Because of the large number of animals to be used in this experiment and the complexity of the injecting procedures, animals were bred in three batches, with equal numbers of animals from the three experimental groups being assigned to each amphetamine group in each of the three batches, thus ensuring counterbalancing.

b) Drug Doses:

In this experiment five dose levels of the drug *d*-amphetamine sulphate, obtained from the Sigma Chemical Company Ltd, were employed. In particular, Dose A, the placebo or baseline dose employed as a control, consisted of 0.0mg/kg *d*-amphetamine; Dose B, 0.5mg/kg; Dose C 1.0mg/kg; Dose D, 1.5mg/kg; and Dose E, 2.0mg/kg. For the four dosages containing the drug, the amphetamine salt was dissolved in physiological saline, with an initial stock solution being prepared expressed in terms of 1ml/kg body weight. From this stock solution, the various doses were obtained by the method of successive dilution. Fresh drug preparations were made up at the beginning of each week [5] and when not in use, kept in a refrigerator. In both experimental tests, drugs were injected intra peritoneally, using 25g 5/8" needles attatched to disposable 1ml syringes.

c) Apparatus:

The open field and the Type I Skinner box system as well as the maternal environments employed in this experiment are detailed in the general methodology chapter (chapter four).

[5] This procedure was recommended by Dr Martin Baxter and had been successfully piloted prior to the commencement of this experiment.

d) Procedure:

At weaning, subjects were weighed and assigned to individual cages prior to the start of testing, at which time a technician recoded all the animals, so as to disguise their experimental backgrounds. Consequently, the only information available to the experimenter was the drug condition of each subject, animals being numbered according to dosage, for example A1, A2 and so forth.

As amphetamine has been found to influence feeding patterns (Grilly 1977) and thus might affect the normal growth of the subjects, it was decided to minimise exposure to the drug until the animals had attained a more robust size and bodyweight. It was decided therefore to run the Skinner box test first, as animals would have at least two weeks without drugs, to encourage healthy development.

The Skinner box procedure employed in this experiment was the same as employed in chapter seven (experiment two) and was based on the work of Rose et al (1986). In particular, following two days of maintenance feeding where the animals were given 8gms of food at the end of each day, subjects began an 18 day Skinner box procedure according to the following sequence of schedules: CRF (Days 1-5); FR3 (Days 8-9); FR6 (Days 10-12 and 15-18). No training was carried out on days 6, 7, 13 and 14, subjects being fed ad libitum on these days. Throughout the experimental procedure subjects were weighed daily to ensure normal growth was maintained. Subjects were run in groups of three, for 30 minute trials and animal running order was randomised on each day of testing.

Reinforcement in this experiment, as in chapter seven, consisted of the sound of the lever microswitch, paired with the sound of the pellet dispenser, supplemented by one second of illumination of the white perspex roof of the operant chamber and a pellet of food. The light level was set at 750 foot lamberts and after each trial number of bar presses and food pellets eaten were recorded and the equipment cleaned and relay counters re-set for the next group of animals.

Following a procedure developed in the author's laboratory (Dell 1985: unpublished data), the

introduction of amphetamine to the procedure was delayed until all the animals had a well established bar press habit. Specifically, the groups were given their appropriate doses of amphetamines on the last three days of testing, Days 16, 17 and 18, the procedure for a typical trial being as follows; half an hour prior to testing the three animals to be run next in the random sequence received their i.p. injections in a 'prep' room away from both the colony room and the experimental rooms and were then left in this room in their home cages until they were required for testing. Having removed the preceding group of animals, taken the appropriate behavioural measures and placed the drugged subjects into the Skinner boxes, the next three experimental animals were injected, and so the procedure continued until all the animals had been tested.

Following the completion of the Skinner box testing, all animal were put onto an ad libitum diet for a week, to allow all traces of the drug to clear the animals' systems. Following this break, at approximately 48 days of age, all the animals underwent five daily three minute trials of open field testing, following a procedure similar to that employed in chapter five, slightly modified to allow the experimenter time to inject the subjects between trials. Because of the need to inject each animal half-an-hour prior to the start of testing, an initial seven animals were injected with their appropriate doses at five minute intervals. Once the first animal to be injected had waited for half-an-hour in the prep room in its home cage, it was removed to the experimental room, placed in the open field and observed for three minutes, during which time the numbers of lines crossed, rears and defecations were recorded [6]. On removal from the field the animal was returned to its home cage, the field wiped down and the eighth animal to be injected given its drug dose. This procedure continued until all the animals were tested, running order being randomised at the start of each day of testing.

[6] In this experiment, time spent in the centre of the open field was not recorded, as the animals were not experimentally naive and therefore less likely to be emotional in the open field.

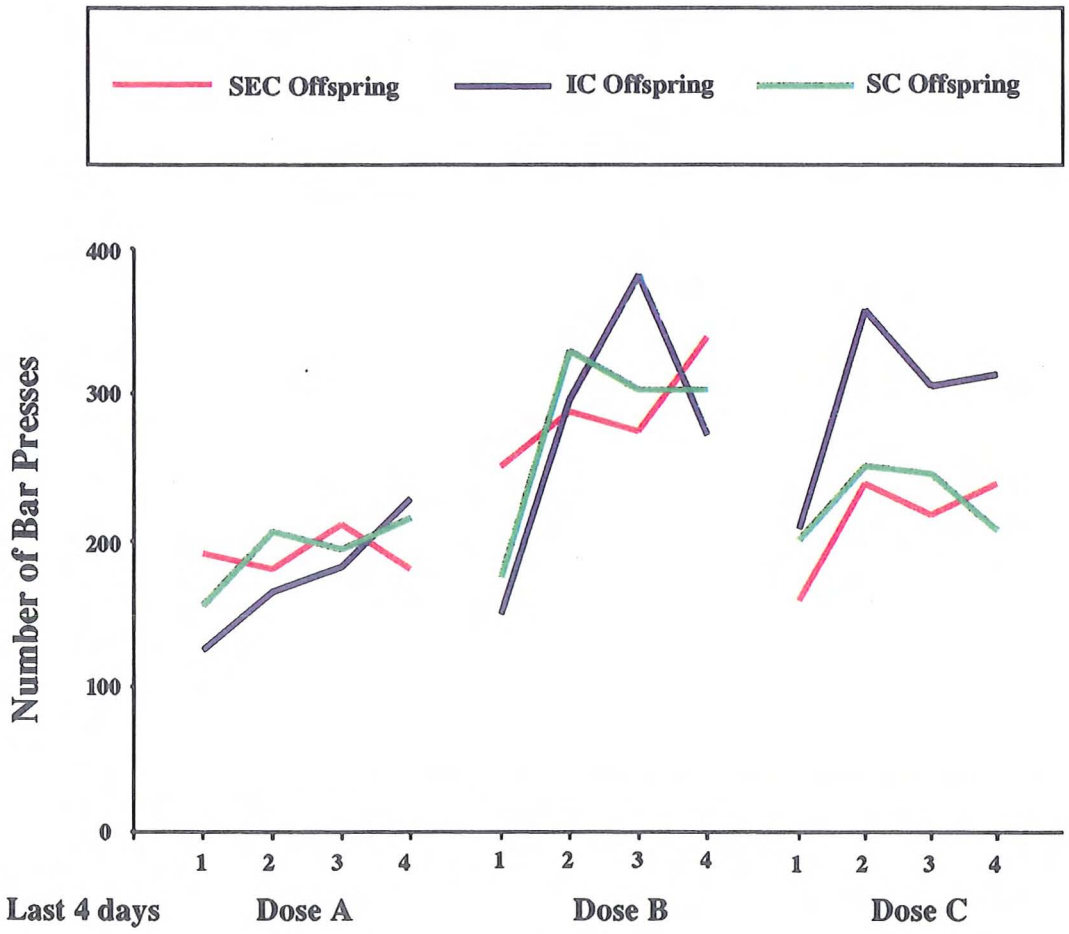
8:3 RESULTS

As with all the previous experiments, before analysing the behavioural data, to ensure that any littersize variations existing between the experimental groups were not significant, a two factor ANOVA of size of litter from which each subject was drawn, was carried out. This failed to reveal any significant differences between either the experimental groups $F(2,75)=0.55$ $p>0.05$, or between the five drug dose groups $F(4,75)=0.35$ $p>0.05$. Furthermore, as was apparent from the lack of significant interaction $F(8,75)=0.41$ $p>0.05$, the experimental subgroups were also equatable with respect to littersize.

a) Skinner Box:

Considering the pre-drug bar press rates first, although offspring of SC mothers bar pressed more than either their SEC or IC counterparts (means: SEC: 185.83; IC: 183.37; SC: 210.73 N=30 per group), analysis of variance of the number of bar presses on day 15 for the three offspring groups revealed no significant differences between their performances $F(2,75)=0.526$ $p>0.05$. Furthermore, there were no differences in performance between the animals that would be assigned to the different dose levels of the drug $F(4,75)=0.891$ $p>0.05$, nor were there any differences between the offspring group by dose level subgroups $F(8,75)=1.085$ $p>0.05$.

In addition to analysing the last day prior to drug administration, the initial eleven days of training (including the last pre-drug day) were also subjected to an analysis of variance to investigate the patterns of performance for the three offspring groups. Predictably there was a highly significant learning effect $F(10,750)=141.57$ $p<0.001$, however, in this experiment no significant differences emerged between the three experimental groups $F(2,75)=0.27$ $p>0.05$, nor was there a significant group by days interaction $F(20,750)=0.53$ $p>0.05$. Furthermore and as would be expected, no differences emerged between the different drug groups $F(4,75)=0.83$ $p>0.05$, nor were there any significant interactions, $F(8,75)=0.85$; $F(40,750)=0.86$; and $F(80,750)=0.97$ $p>0.05$,



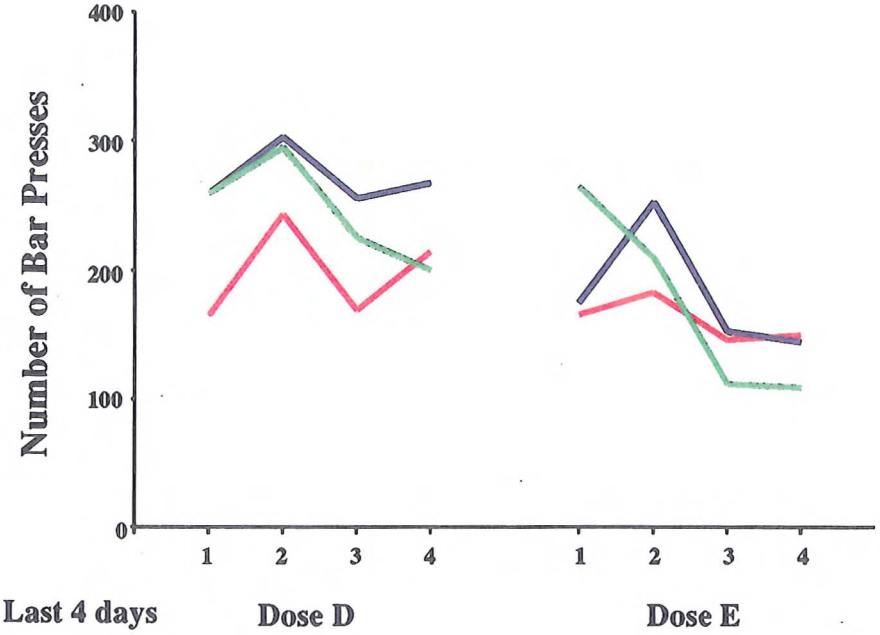


Figure 8.1

Mean number of bar presses by the three offspring groups over the last four days of Skinner box testing, for the five drug dose levels. (A=0.0mg/kg: B=0.5mg/kg: C=1.0mg/kg: D=1.5mg/kg: E=2.0mg/kg)

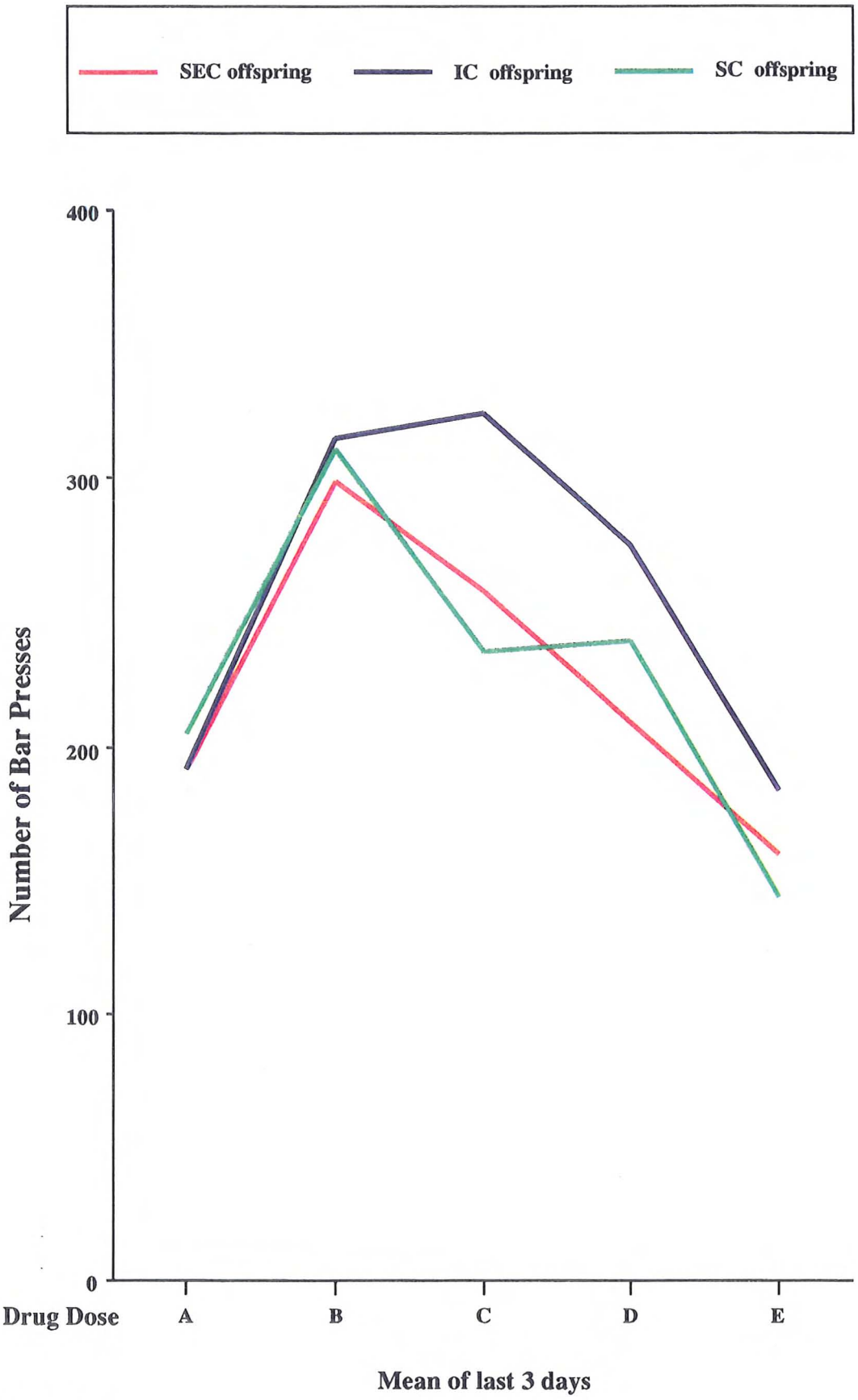


Figure 8:2

Mean number of bar presses by the three offspring groups for the last three days of testing, for the five drug dose levels.
(A=0.0mg/kg: B=0.5mg/kg: C=1.0mg/kg: D=1.5mg/kg: E=2.0mg/kg)

for experimental group by dose, days by dose, and group by days by dose respectively.

Moving on to the last days of training, during which time animals were given their respective drug doses, as can be seen from Figure 8:1, which describes the mean number of bar presses of the three offspring groups over the last *four* days of Skinner box testing (last pre-drug day and three days with drugs) in the drugged subgroups (B, C, D and E), IC offspring tended to bar press more than their SEC and SC counterparts. This is particularily evident from Figure 8:2, which describes the means of the three offspring groups over the last three days of testing, when the *d*-amphetamine sulphate was administered. An analysis of variance of the number of bar presses per day over the last three days of testing (during which time amphetamine was administered), for the three offspring groups, however, failed to reveal any statistically significant differences between the experimental groups $F(2,75)=0.63$ $p>0.05$, although there was a significant effect due to dose level $F(4,75)=2.89$ $p<0.03$. As can be seen from Figure 8:2, the highest bar press rates occured under dose level B (0.5mg/kg of *d*-amphetamine sulphate), with the higher dose levels gradually reducing all three groups' bar press rates. Although there was a tendency for IC offspring to maintain higher levels of bar pressing for dose levels C and D (1.0mg/kg and 1.5mg/kg), this was not statistically significant there being no significant group by dose level interaction $F(8,75)=0.16$ $p>0.05$. Finally, as can be seen from Figure 8:1, there was a significant days effect $F(2,150)=3.41$ $p<0.04$ but the days by dose effect $F(8,150)=1.52$ $p>0.05$ and the group by dose by days $F(16,150)=0.63$ $p>0.05$ interactions were non significant suggesting that the dose-response curves for the three offspring groups were similar.

b) Open Field:

As with the Skinner box data, ANOVAs of the open field dependant variables revealed no significant differences between the three experimental groups $F(2,75)=0.98$, 1.12 and 0.65 $p>0.05$ for number of lines crossed, rears and defecations respectively. Furthermore, there were no significant differences in dose levels for either the lines crossed measure $F(4,75)=1.25$ $p>0.05$ or

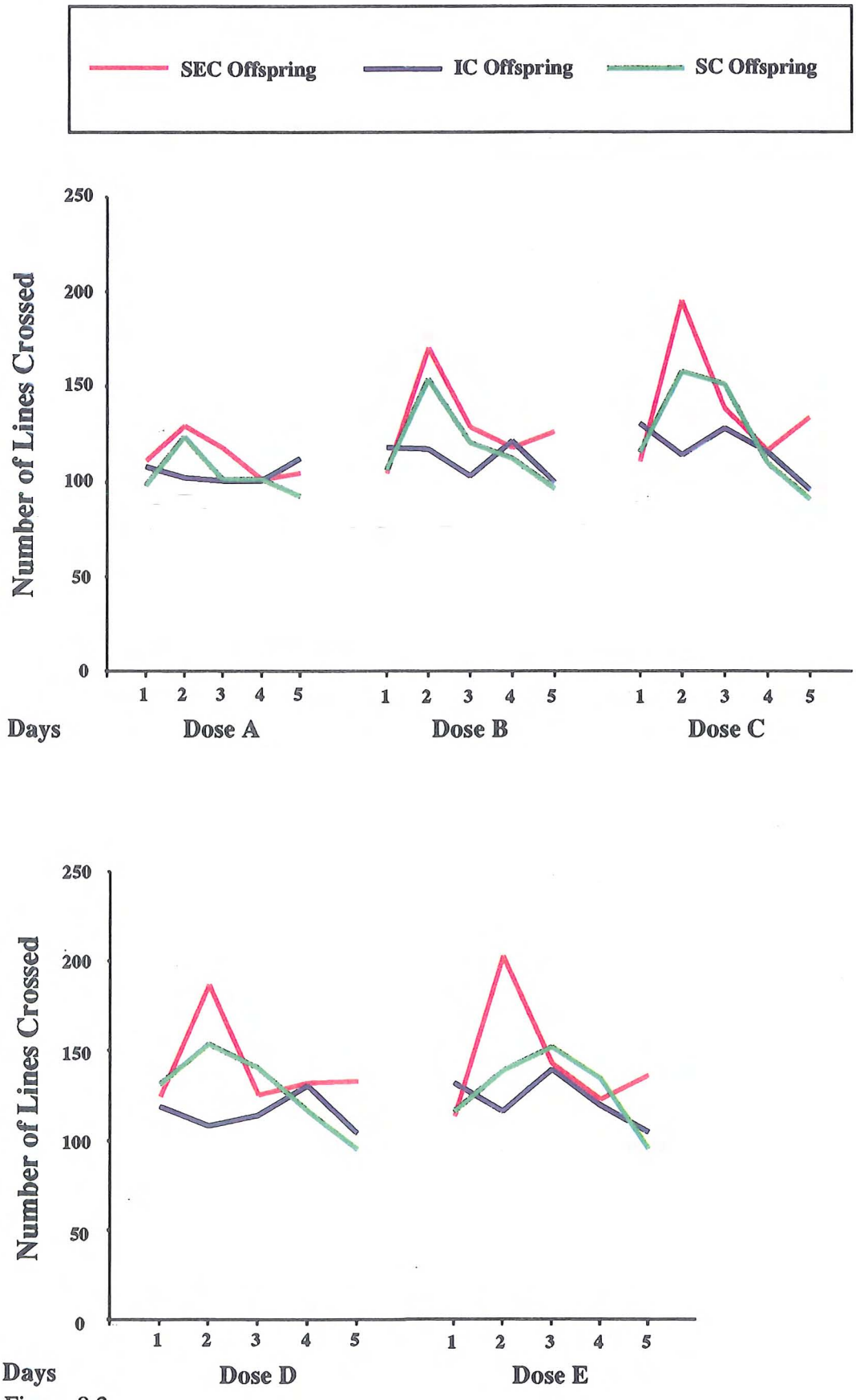


Figure 8.3

Mean number of lines crossed by the three offspring groups over the five days of open field testing, for the five dose groups.
(A=0.0mg/kg: B=0.5mg/kg: C=1.0mg/kg: D=1.5mg/kg: E=2.0mg/kg)

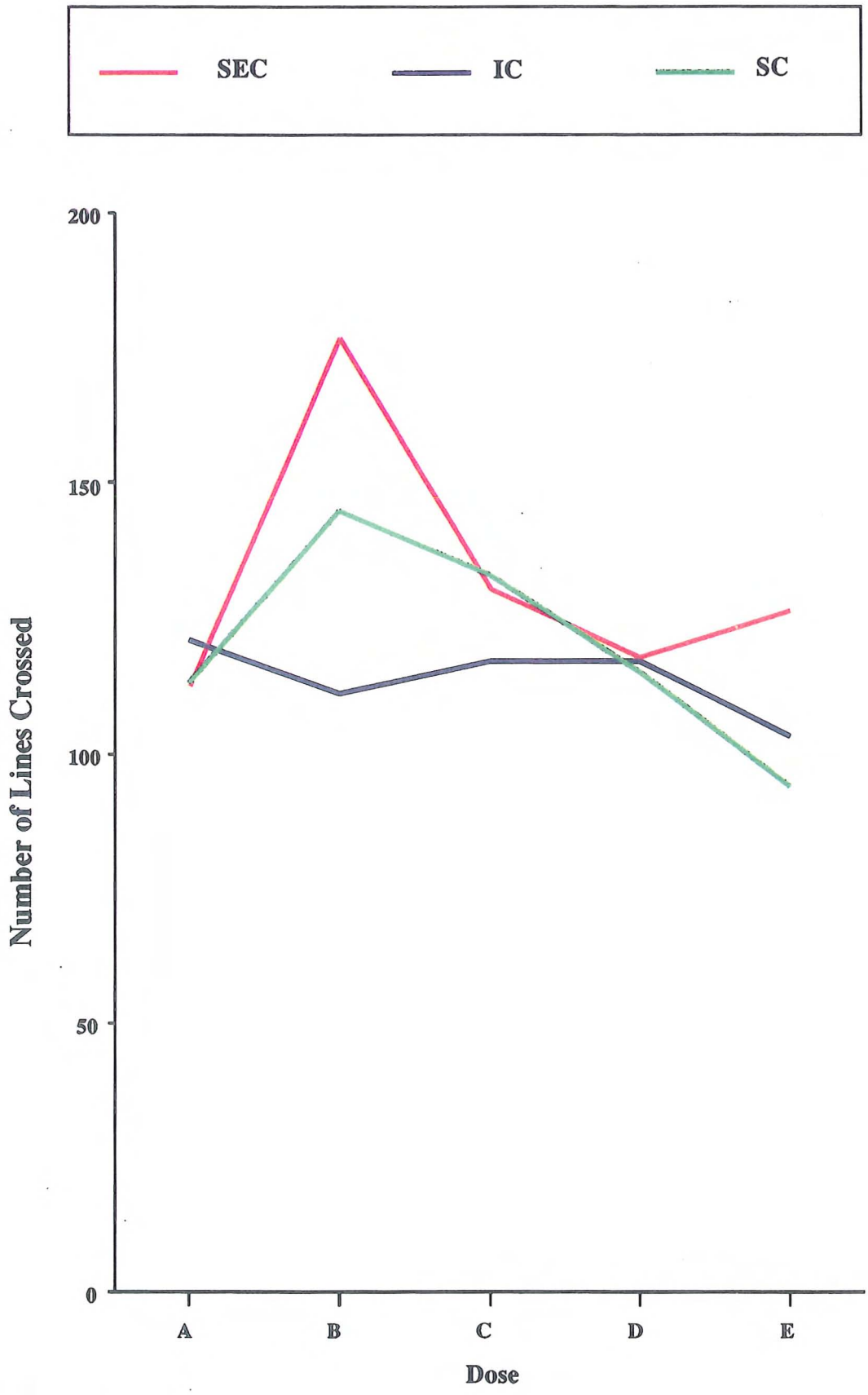


Figure 8:4

Mean number of lines crossed per drug dose
by the three offspring groups.
(A=0.0mg/kg: B=0.5mg/kg: C=1.0mg/kg: D=1.5mg/kg: E=2.0mg/kg)

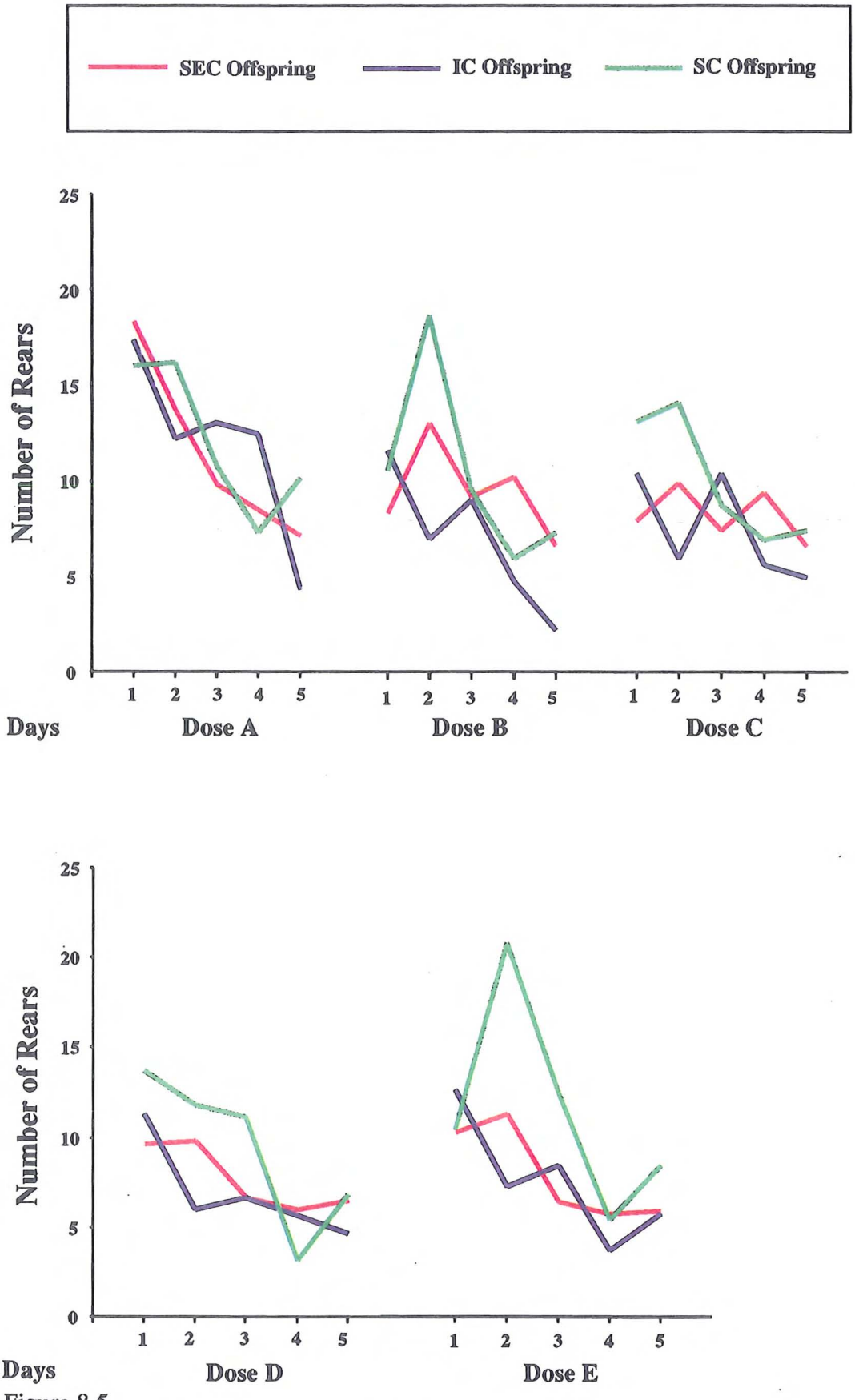


Figure 8.5

Mean number of rears by the three offspring groups over the five days of open field testing, for the five dose levels. (A=0.0mg/kg: B=0.5mg/kg: C=1.0mg/kg: D=1.5mg/kg: E=2.0mg/kg)

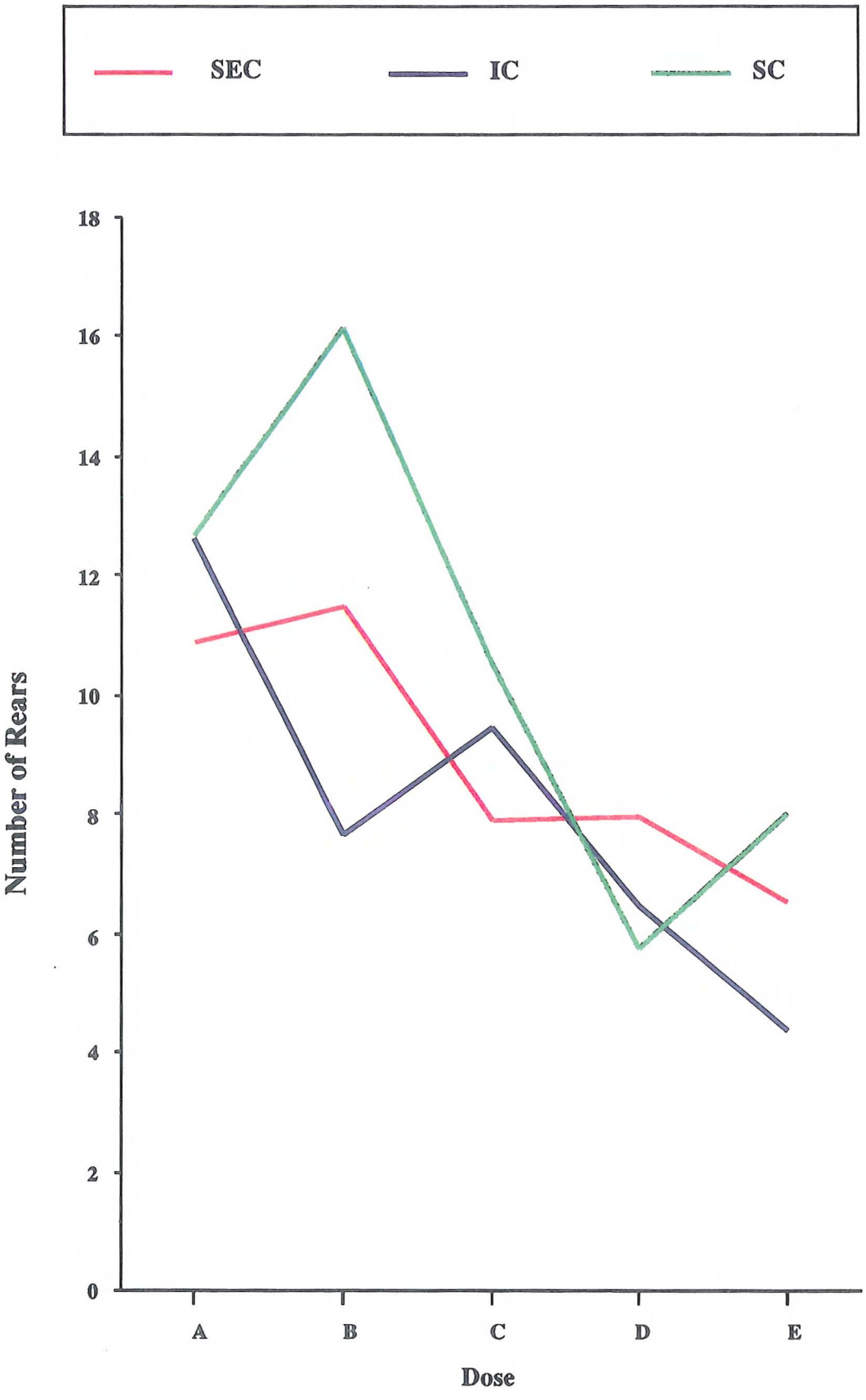


Figure 8:6

Mean number of rears per drug dose
by the three offspring groups.
(A=0.0mg/kg: B=0.5mg/kg: C=1.0mg/kg: D=1.5mg/kg: E=2.0mg/kg)

the defecation measure $F(4,75)=0.84$ $p>0.05$. Different doses of *d*-amphetamine sulphate did however produce significant effects in the number of rears measure, $F(4,75)=2.98$ $p<0.02$, with a gradual reduction in number of rears occuring as the dose level increased (means: Dose A: 12.06; Dose B: 11.76; Dose C: 9.28; Dose D: 6.70; Dose E: 6.30). The third main effect to be investigated, the repeated measure, days, predictably was highly significant on all three behavioural measures $F(4,300)=10.90$, 9.70 $p<0.001$ and 2.53 $p<0.03$ for lines crossed, rears and defecations respectively.

Of particular interest to the present thesis, however, were the dose by groups interactions. In particular, it was predicted that by increasing SEC and SC arousal levels, their performances should resemble those of the IC offspring. As can be seen from Figures 8:3 and 8:5 which describe the means of the three offspring groups over the five days of testing in the five dose groups, for lines crossed and rears respectively, there was a tendency for SEC offspring to cross more lines and for SC offspring to rear more than their IC counterparts in most of the drugged trials. (See also drug-dose response curves for lines crossed and rears, Figures 8:4 and 8:6 respectively). Unfortunately, however, this was not found to be statistically significant $F(32,300)=0.79$, 0.84 and 0.87 $p>0.05$ for the number of lines crossed, rears and defecation measures respectively. Moreover, none of the other interactions was significant.

8:4 DISCUSSION

In the present experiment the possibility that the differential behavioural profiles observed in SEC, SC and IC offspring in the previous two studies reflected underlying differences in baseline arousal levels was examined, by chemically altering the subjects' arousal levels with *d*-amphatamine sulphate. Results failed to find any statistically significant differences between the groups' dose-response patterns, suggesting that the observed group differences cannot be meaningfully described in terms of a unitary intervening variable such as arousal. Indeed, given that Einon and Sahakian (1979) have argued that there is a complex interaction between expe-

riential background, gender, diurnal variation and drug dose, postulating arousal as a causal or intervening variable does appear to be "too simplistic an explanation" (p300).

a) Offspring Skinner Box Performance

In the introduction, it was suggested that offspring pre-drug bar press rates over the last day of testing prior to drug administration should differentiate between the offspring groups, such that SEC and SC progeny should bar press more than their IC counterparts, paralleling the findings reported in chapter seven (experiment two). Analysis of this data failed to reveal any significant differences between the groups. Futhermore, when offspring performances over all the pre-drug training days were analysed, no significant differences in learning curves for the three offspring groups emerged. In the previous study, however, significant differences did emerge between the three offspring groups. One way to attempt to explain the discrepancies in findings between the two studies is to focus on the differences in procedures that were employed. In particular, in the previous study, offspring groups' performance levels were analysed when they had reached a high level of bar pressing, that is after eighteen days of training. In this study, offspring levels of performance were measured at an earlier stage in the acquisition phase (day fifteen), perhaps before any differences might reasonably be expected to have manifested themselves. It might be that offspring learning profiles only start to be differentiated at the higher bar press levels associated with the last few days of training. However, consideration of Figure 7:7 does not support this idea, as by day fifteen in the previous study the three offspring groups' learning curves were already progressing at different rates. This suggests that whatever is causing the differences between the studies, measurements taken at different stages in the procedures cannot be the answer.

Furthermore, when considering the behaviour of the saline group in this experiment, the lack of group differences being attributed to pre-asymtotic measurement can no longer be considered a suitable explanation for the findings. At this stage in the procedure, offspring should have

achieved a similar level of performance as the animals in the preceding study. It was predicted that with these animals (subjected to injections but not administered any drugs) the SEC and SC offspring groups should bar press more than their IC counterparts. As can be seen from Figure 8:1, this was not the case. Indeed, all the animals' levels of bar pressing were remarkably similar. However, it should be noted that the experience of being restrained and then injected is not pleasant and might even be considered arousal-inducing (Curry 1987). Indeed, this procedure might well have aroused the offspring groups, reducing the bar press levels of the SEC and SC animals to those of their IC counterparts [7]. Considering the mean levels of bar pressing of the saline groups, when compared to those of the animals in the previous study, as can be seen from Table 8:1, in the present study both the SEC and SC offspring have lower bar press rates than were observed in the previous study. This adds some credence to the hypothesis that, as with Einon and Sahakian's (1979) saline groups, the injection procedure has an impact on the offspring groups too. Interestingly, however, the reverse is true for the IC offspring in that these animals produce considerably higher performances when given saline injections than when not.

STUDY	DAY	SEC	SC	IC
Experiment	15	204.21	211.32	109.89
Two	16	259.47	240.37	114.26
Chapter	17	274.21	246.79	148.42
Seven	18	260.42	259.53	160.63
Present	15	191.33	155.83	125.33
Experiment	16	181.16	206.0	164.66
	17	210.83	194.0	181.5
	18	181.16	215.16	229.16

Table 8:1 Means of the offspring groups Skinner box performance over the last four days of testing, for the experimental animals employed in chapter seven (N=19 per group) and the animals used in the saline condition in the present study (N=6 per group).

In the introduction, it was further suggested that as the dose levels of amphetamine increased,

[7] Einon and Sahakian (1979), for example, have found evidence that the injection procedure does affect animals' behaviour. In their study, isolated rats demonstrated more stereotypic behaviour than socially reared animals following saline injections.

as would be predicted by the literature (Grilly 1977), animals bar press rates should increase initially, then decrease with the higher doses. As can be seen from Figure 8:2, this was the case. In addition, it was suggested that the dose-performance curves of the three offspring groups should differ, with IC animals reaching an asymptotic level at lower doses than their SC and SEC counterparts. This was not the case. This latter finding is of particular importance to the present study, so it will be dealt with in some more detail.

As mentioned earlier, from the second experiment reported in chapter seven, it was suggested that IC offsprings' low bar press rates, compared with their SEC and SC counterparts, could perhaps be explained in terms of their being overstimulated by the high levels of illumination which formed part of the complex reinforcer. This would raise their arousal levels, resulting in lower performance, following the Yerkes-Dodson law (1908). In the present work, it was therefore hypothesised that by increasing SEC and SC offspring arousal levels, their performance should be gradually reduced to IC levels, as the drug dosage was increased. As can be seen from Figure 8:2, which describes the experimental groups by drug dose interaction, baseline performance levels in the saline condition (Dose A) were very similar for the three experimental groups, all of whose bar press rates increased in parallel for drug dose B, (0.5mg/kg). For the higher drug doses, the picture initially appears even more confusing. Taking the performance of the SEC and SC offspring groups first, as predicted by the arousal hypothesis, both groups' bar press rates fell off as the drug dose was increased. With the IC offspring groups, however, although not statistically significantly different from SEC and SC offspring, bar press rates remained high for drug doses C and D (1.0mg/kg and 1.5mg/kg), before being reduced by the highest dose level, dose E (2.0mg/kg).

This deviation in the IC offsprings' performance, coupled with the lack of statistically significant differences between the three groups' dose-response curves may, however, reflect large inter-subject variance. Indeed, *d*-amphetamine given intraperitoneally to albino rats at various doses has been found to produce different results depending on the subject (Will and Checchinato

1973). In their study amphetamine was found to increase response rates in some animals and decrease it in others. Because the present study did not employ a repeated measures design with respect to drug dose, nor was the drug administration maintained for a long period of time (only three doses being administered in this experiment rather than five doses over a period of a fortnight in Will and Checchinato's study) it is not possible to examine the present findings on an individual-rat basis. However, individual differences in autonomic reactivity might well be worth investigating in a future study.

To summarise the Skinner box findings, several interesting points have emerged. Firstly, unlike the previous Skinner box experiment (chapter seven) the present experiment failed to reveal any significant differences between the offspring groups' bar press rates prior to drug administration. It was suggested initially that this might have reflected the pre-asymptotic performance of the groups and that offspring differences might have occured if the training procedure had been maintained for a longer period. However, comparison of the present findings with the previous study did not offer any support for this hypothesis. Secondly, no significant differences emerged between the groups over the four days of testing under the saline condition, although there was a tendency for IC offspring to bar press more than their SEC and SC counterparts. This finding is in direct opposition to that predicted by the arousal model. Finally, as was predicted, all offspring performances were affected by the dose of drug, such that the lower doses of drug increased performances across the board and higher doses of drugs decreased performances.

b) Offspring Open Field Performance

Moving on to the second test employed in this study, the open field, it was predicted that offspring groups would differ in their dose-response curves over the five days of testing, in that if the IC progeny were more aroused then they would reduce their levels of activity at lower doses than either their SEC or SC counterparts, the latter groups' profiles reaching a ceiling at the higher doses of *d*-amphetamine. Saline groups' performances should be similar to those of the animals

tested in chapter six.

Considering the dose-response curves first, although there were no significant differences between the groups' patterns of responding over days for the five dose levels for the lines crossed or number of rears measures, as can be seen from Figures 8:3 and 8:5, SEC and SC animals tended to be more active than their IC counterparts respectively, which is consistent with the experimental hypothesis. However, with the higher dose levels these animals were still highly active, rather than reducing their levels of performance as was predicted. It may well be that for these animals, having already been highly trained in the Skinner box task, the "novelty" of the open field was quickly habituated to and thus this apparatus was not arousal-inducing. Therefore, the cumulative effects of the novelty of the field and the injections may have been such as to raise these animals' arousal levels towards the asymtote, but not to reach the ceiling effect that was predicted. Interestingly, when considering Figure 8:4, IC offspring do not seem to be affected by the drug dose at all, their mean number of lines crossed hardly changing over the five drug doses, unlike their SEC and SC counterparts. Their rearing behaviours, however, do seem to respond to drug doses, suggesting that the drug preparations were not at fault. Why there is a discrepancy between these measures, is at present unclear.

With the number of rears, the only significant pattern to emerge was that for all the offspring groups, as dose levels increased number of rears decreased. Furthermore there was a highly significant days effect, animals tending to reduce their rearing behaviour as they habituated to the apparatus over the five days of testing. Neither of these results are surprising. What is unusual, however, is the lack of significant dose-response differences between the groups. It was predicted that if the IC progeny were more aroused than the other two groups, then they would reduce their activity more quickly than either the SEC and SC offspring. In fact all the groups reacted in a similar way to the drug doses as evidenced by the lack of significant drug dose by offspring group interaction. However, it has been noted (Randrup and Munkvad 1970) that the various behavioural elements asociated with stereotypy are differentially affected by

amphetamine as the drug takes its course. It may be, therefore, that rearing and locomotion are being differentially influenced by environment and drug dose in the present experiment but that at the time the animals' behaviours were measured (namely for three minutes, half an hour after injection), the effects were not present.

Finally, considering the saline dose group, it was suggested that these animals' performances should be similar to the pattern of responding observed in the SEC, IC and SC progeny in chapter six (experiment one). In particular, in the earlier study, although no significant differences emerged between the three offspring groups as a main effect, patterns of responding over days were different; SC offspring were more active than their IC counterparts, these animals being more active than the SEC progeny, both the latter groups tending to increase their activity over days. In the present study, although no significant interactions emerged, SEC and SC animals tended to be more active than than the IC group, when saline was injected. Perhaps this reflects the beginnings of an arousal-induced performance in these animals and that to expect the offspring groups' open field behaviours to be similar whether or not they had been injected is inadvisable.

c) Summary and Conclusions

In the present experiment the *nature* of the behavioural differences observed between the offspring groups in previous experiments was investigated. In particular, it was argued that if the differences noted between the offspring performances in any way reflected differential positions on an inverted 'U' shaped performance-arousal curve, then certain predictions could be made about the offspring groups' behaviour under chemically altered states of arousal. Despite the lack of statistically significant differences between the three offspring groups, there were tendencies in the data which warranted some discussion. Overall, however, the present data did not support the view that offspring of differentially housed animals were differentially aroused. However, as cautioned by Einon and Sahakian (1979), observed differences in activity between different groups of animals "can only be used as supporting evidence for arousal differences; it does not

of itself constitute strong evidence" (p306). Obviously this is an area of research which warrants more investigation, a fact that will be discussed in more detail in the following chapter.

CHAPTER NINE: GENERAL DISCUSSION

9:1 INTRODUCTION

The effects of exposing an animal to an enriched environment are now well documented and it is apparent from the numerous studies that have emerged in the literature over the last four decades that enrichment produces an animal which is qualitatively different from either its socially housed or impoverished littermates. In particular, as outlined in chapter two, the enriched environment engenders in its inhabitants an ability to adapt more efficiently to new situations and affords these animals enhanced opportunities for learning skills which according to Renner (1988) have a survival function. Effects of enrichment have been found at a neuroanatomical, neurochemical and behavioural level (see chapters one and two) and in recent years have been extended beyond the laboratory to change the ways in which a variety of social and economic issues are now addressed (Rosenzweig 1984).

As has become apparent from this thesis, however, the effects of enrichment need not be confined to those directly exposed to it. Indeed, as noted in chapter one, in the orient and particularly in Japan, there is a widespread belief that cultural enrichment can extend across generations via intruterine education, a procedure known as Taikyo (Nakae 1983). Within the enrichment literature, there has been a handful of studies which have experimentally investigated this phenomenon by exposing pregnant animals to EC, SC and IC and investigating the effects of these environments on both brain measures (Diamond et al 1984; Inouye et al 1986) and some behaviours (McKim and Thompson 1975; Kiyono et al 1985) in their offspring. However, as yet little is known about the functional significance of these effects or even their adaptive value. The present thesis aimed to explore this area of research further by both examining the effects of differential environments *prior* to pregnancy across three successive generations and beginning to unravel the nature of these effects in more detail.

In this chapter the aims and objectives of this thesis will be reiterated and a summary of the main findings provided. In addition, methodological problems that might influence the interpretation of the results will be highlighted. Possible causes of the effects observed will then be discussed

and avenues for future research suggested. Finally, implications of the findings will be mentioned and conclusions based on this research drawn.

9:2 AIMS, OBJECTIVES AND SUMMARY OF FINDINGS

As outlined in the General Introduction (chapter one) to this thesis, the purpose of the present research was to investigate the effects of differential maternal environments prior to pregnancy on future offspring. In particular, the experimental programme was designed to explore three areas of interest.

- Firstly, to investigate the effects of exposing animals directly to SEC, SC and IC on their behaviour, both to validate the use of the superenriched environment employed in this present work and to ensure that environmental effects continued postpartum.
- Secondly, to establish a behavioural profile of offspring and grandoffspring of differentially housed mothers, that is to investigate the intergenerational transfer of environmental effects.
- Finally, to investigate the possible causes of the intergenerational effects, with a more detailed analysis of offspring performances being undertaken in both learning and activity based tasks.

Given these aims, the first issue to be addressed in this discussion, is whether they were realised or not. This will become apparent as the main findings of this thesis are described in more detail below.

9:2:1 BEHAVIOURAL PROFILE OF SEC, SC AND IC ANIMALS

The first aim of this thesis was to provide a behavioural profile of animals exposed directly to the differential environments. This was undertaken for several reasons:

- Firstly, to ensure that the superenriched environment employed in this thesis provided a sufficiently enriching experience compared with the impoverished environments, given the length of time for which animals would be maintained in it. In addition, by including a standard group housing condition (SC), the relative contribution of SEC versus IC to performance differences could be assessed.
- The second objective was to establish a behavioural profile of male and female animals exposed to SEC, SC and IC against which to compare offspring and grandoffspring groups.
- Thirdly, as the experimental manipulation of the mothers employed in this thesis occured prior to pregnancy, it was considered important to establish that the environmentally induced changes in behaviour continued post partum without being reduced by pregnancy and the rearing of offspring.

These objectives were incorporated into the design of the first study of this thesis (chapter five), the underlying rationales and findings of which are discussed in the following pages.

a) Efficacy of Superenriched Environment

In the present research, manipulation of the maternal generation by exposing female animals to SEC, SC and IC from weaning to maturity resulted in animals being differentially housed for nine weeks. Given that during this length of time animals might habituate to a more traditional form of enrichment (such as has been typically employed in the EC/IC literature) and given that on balance more studies have found beneficial effects of superenrichment (Sturgeon and Reid 1971; Kuenzle and Knusel 1974; Bennett 1976) than not, in the present thesis it was decided

to employ a superenriched environment to maximise enrichment effects. As a consequence of this, it was necessary to establish clear differences between animals raised in SEC, SC and IC, before employing these environments as maternal manipulations in subsequent experiments. Consequently, the effects of these environments on animals' open field, visual cliff and Skinner box performances were investigated in study one of this thesis (chapter five).

In the open field, significant differences were found between the three experimental groups, IC animals crossing more lines than either their SEC or SC counterparts over the last four days of testing. This main effect was qualified by a significant environment by days interaction such that IC animals and in particular female IC animals maintained higher levels of responding over days than their SEC and SC counterparts. Significant interactions involving sex of animal also emerged, which when investigated further revealed that for the lines crossed measure, all significant group effects were due to the female animals. With the rearing measure, although SEC animals reared more than IC and SC animals, group profiles over days also varied, SC animals' number of rears reducing at a faster rate over days than their SEC and IC counterparts. No significant differences emerged between the groups with respect to either the time in centre or number of defecations measures, although males were found to defecate more than females.

With the Skinner box data, clear differences emerged between the SEC and IC groups, irrespective of whether the animals were male or female. IC animals bar pressed significantly more than their SEC counterparts. With the SC group, however, profiles relative to the SEC and IC animals varied according to sex, SC females mirroring SEC performances, SC males those of their IC counterparts. Finally, with the visual cliff data, where both latency to descend onto the cliff and side chosen were measured, SEC animals took less time to descend onto the cliff than their SC and IC conspecifics, irrespective of cliff depth. Futhermore, although both IC and SC groups appeared more likely to pick the shallow side of the cliff, when the deep side was set at twelve inches, the sample sizes precluded an adequate statistical analysis of these data.

Considering the efficacy of the superenriched environment, clear SEC/IC differences were found

in all three behavioural tasks, albeit qualified by the sex of the animals. Furthermore, in most instances these differences paralleled those typically found in studies employing the more traditional type of EC. Starting with the open field results, IC animals demonstrated higher levels of activity than their SEC counterparts, a pattern of increased ambulation that has been reported elsewhere in the literature (Woods et al 1960; Dell and Rose 1987). Moreover, IC animals were more active than their SC counterparts, suggesting that ambulation differences were due to IC response patterns being elevated, rather than SEC levels being depressed. This SC/IC difference is consistent with work by Syme (1973) Einon et al (1975; 1978) and Morgan and Einon (1976), all of whom have reported their IC animals to be more active than socially housed animals. With respect to the rearing mesaure, SEC animals reared more than their IC and SC counterparts, demonstrating that the superenriched environment employed in this thesis produced animals with qualitatively different behavioural profiles than the SC and IC environments in this measure. No differences were found between the groups, however, with respect to either amount of time spent in the centre circle or number of defecations.

At this point it is worth mentioning the sex differences found in the patterns of responding in the lines crossed measure of the open field. The higher levels of IC responding in the female groups when compared with their IC males and SEC male and female counterparts, is not entirely unexpected. Woods et al (1960) have also found larger EC-IC differences in female animals when compared with male groups. These gender differences are not often found in the literature, however, as the majority of studies has typically only employed either male or female subjects. Of particular interest to the present thesis, is whether or not the lack of significant differences between the male groups can be attributed to the SEC. Consideration of the means of the groups suggest that it is the IC males' performances which are unusual, reducing their activity over days rather then maintaining their more usual high levels of responding over days. Consequently this result was taken as further evidence of the ability of the SEC employed in this research to produce animals which are qualitatively similar to animals raised in a more traditional EC and thus justifies its use as a maternal environment.

With the Skinner box data, again clear SEC/IC differences emerged. This time, however, both the male and female IC animals bar pressed more than their SEC counterparts, a pattern of responding which has been reported elsewhere in the literature (Coburn and Tarte 1976; Lamden and Rose 1979; Rose et al 1985; 1986; 1987). The relative contribution of enrichment or impoverishment to this finding, however, is less clearly determined than in the other behavioural measures, being confounded by gender. In particular, compared with their SC counterparts, SEC/IC differences in bar pressing appear to be due to the higher IC rates in female animals and lower SEC rates in male animals. No obvious reason has emerged for these sexually dimorphic responses, although it appears that female animals are being more influenced by impoverishment than their male counterparts (cf IC females' lines crossed behaviours too). With respect to the visual cliff data, again significant group differences emerged SEC animals taking less time to descend onto the cliff than both their IC and SC counterparts. This quicker descent of the SEC group when compared to their IC counterparts is similar to Curry's (1987) work with EC animals. In his experiment, IC animals took longer to descend onto the cliff (irrespective of side chosen) than their EC littermates.

These findings, coupled with those of the open field, were taken to indicate that the use of a superenriched environment in the present thesis was justified. It should also be noted at this point, however, that the lack of differences observed between the groups in the lines crossed measure were only noted in the *male* groups. Female animals were significantly different from each other. As the intergenerational investigation in the present thesis only involved manipulating female animals (the mothers of future generations) by raising them in differential environments, the lack of differences between the males in one subset of data was rendered even less important in the context of justifying the use of the superenriched environment. Male differences are more relevant, however, when the profiles of animals raised in the differential environments are described in the next section.

Finally, the present results allow some comments to be made about the relative contribution

of SEC to the behavioural differences observed in this study. When looking at the pattern of performances across behavioural test situations, impoverishment seems to be contributing most to the SEC/IC differences in lines crossed behaviours, producing more active animals and to the females' bar pressing responses, producing higher rates of responding in these animals. Enrichment, on the other hand, appears to contribute most to the rearing behaviour, SEC animals being more exploratory than their SC and IC counterparts, to male bar press rates (SEC animals bar pressing less than their IC counterparts) and visual cliff group differences. These results are generally in accordance with previous work, impoverishment often producing animals with higher levels of activity, enrichment enhancing exploratory and perceptual skills (see summary, chapter two). There is, however, no obvious reason for the sexually dimorphic behaviours observed in the bar press responses.

b) Profile of SEC, SC and IC animals

The second objective of study one was to provide a behavioural profile of the animals exposed to the differential environments employed in the present thesis against which to compare offspring and grandoffspring generations' behaviour. This objective was also achieved and from the data reported above certain characteristics can be identified which distinguish between SEC, SC and IC animals.

Considering the SEC animals first, when introduced to a novel environment such as the open field, these animals were initially reactive, their day one lines crossed performance being significantly higher than those of their SC or IC counterparts. However, these animals habituated to the apparatus, gradually reducing their number of lines crossed over days. This initial reactivity was also apparent in the visual cliff apparatus, SEC animals taking far less time to descend onto the cliff from a central barrier than the other two groups. This reactivity in the SEC group must, however, be seen in the context of the test situations employed. Neither the visual cliff

apparatus nor the open field can be seen as more stimulating than the SEC environment [1]. As has been noted elsewhere (chapter two section 2:4) reactivity is relative, animals tending to be more cautious in situations that are more stimulating than their home cage (Lore 1968) whilst more reactive initially in novel environments of a similar level of complexity to their home environment [2]. The test situations employed in this study can be seen as novel for the SEC animals but they habituated quickly to them, reducing their activity levels. Interestingly, in this experiment, SEC animals did not appear to show any evidence of depth perception, as they demonstrated little concern for the side chosen. However, it should be noted that in their environment these animals were well used to jumping down from ramps and had probably learned that such a drop was not particularly dangerous [3]. Moving on to the last behaviour measured, SEC animals in this study, as in previous work (Rose et al 1985; 1986; 1987) bar pressed less than their IC counterparts. Whether this is evidence of learning differences between the groups, or as has been discussed elsewhere (chapter two) just reflects the higher activity levels of the IC animals (Coburn and Tarte 1976) is questionable.

With the IC animals, a different behavioural pattern emerged, qualified by sex of animal. In particular, female IC rats were initially cautious in the open field compared with their SEC and SC counterparts. However, over days these animals maintained high levels of responding, as measured by number of lines crossed. In male IC animals, however, the higher levels of responding were only apparent on days two and three of testing, activity patterns dropping over the last two days of testing. The reasons for these sexually dimorphic patterns of responding are as yet unclear, but suggest that the effects of impoverishment are stronger in the female IC animal, than in their male counterparts. This idea is born out by the effects of exposure to IC being the main contributer to the EC/IC differences in bar pressing in the female animals, but not in their male

[1] The superenriched environment employed in this thesis can be seen as comprising both open areas such as would be found in an open field and sharp drops such as would be found in the visual cliff.

[2] It should be noted that all animals were tested from individual cages and as such, it might be argued, should not differ in behaviour. The fact that they do, suggests that environmental experiences are cumulative and that the impact of impoverishing the SC and SEC animals by maintaining them in isolation during the testing phase of this experiment should be seen in the context of a longer time framework.

[3] Indeed, the higher rearing levels of these animals appears to corroborate this, suggesting as it does, that these animals are more exploratory than their SC and IC counterparts.

conspecifics. In the visual cliff apparatus, which can be seen as more complex than these animals' home cages, both male and female IC animals took their time before descending onto the visual cliff. Furthermore, when the cliff was set at twelve inches, 90% of these animals chose the shallow side compared with 55% when the deep side was set at one inch. This is not unexpected: these animals having no previous experience of depth would naturally be more cautious. This result does suggest, however, that these animals were able to perceive depth. With the Skinner box, both IC males and females bar pressed more than their SEC counterparts. This is now fairly typical in the literature although the exact causes of this pattern are as yet unknown.

With the SC animals, whose environmental experiences are more complex than those of the IC group but less so than the SEC group, activity patterns were similar to those of the SEC group in the open field, that is initally reactive then over days reducing their number of lines crossed, but more like the IC group in the visual cliff. In this apparatus, not unexpectedly given their home environment, SC animals demonstrated a degree of caution, taking longer to descend onto the cliff than their SEC counterparts. In addition, as with the IC animals, a greater proportion of animals chose the shallow side (80%) when the cliff was set at twelve inches than when it was set at one inch (60%). With the Skinner box data, however, sex of animals interacted with performance. Male SC animals' bar press rates mirrored the IC males performances, female animals the SEC females' performances. As mentioned earlier, reasons for these sexually dimorphic behaviours are as yet unknown.

As can be seen from this section, direct exposure to SEC, SC and IC produces animals with different behavioural profiles. Furthermore, these profiles are not dissimilar to those found in previous studies investigating EC, SC and IC open field, visual cliff and Skinner box behaviour, summarised in some detail in chapter two (section 2:4).

c) Effects Continue Postpartum

The final objective of study one was to ensure that the differences observed between the SEC, SC and IC groups noted in the previous two sections continued postpartum. The rationale behind this was quite simple. In the experimental procedure employed in chapters six, seven and eight, manipulation of the maternal generation by exposing females to differential environments was to be carried out. If the behavioural effects induced by direct exposure to these environments were to be masked by the experiences of pregnancy and associated prenatal individual housing coupled with the raising of litters, then the use of this form of maternal experience would be called into question. Consequently, in the second experiment in study one, females which had succesfully undergone environmental exposure, pregnancy and litter rearing were tested in the open field.

Results indicated that significant differences existed between the three postpartum groups. Over the five days of testing, SC and IC groups maintained higher levels of activity than their SEC counterparts as measured by the number of lines crossed and were more exploratory as seen by their increased number of rears. Animals raised in the SEC prior to pregnancy spent less time in the centre of the open field compared with their IC littermates. However, when the relative performances of the postpartum groups are compared with their virgin counterparts' behaviour, several differences between the two groups of animals' patterns of responding emerged.

In particular, although both the virgin and postpartum IC animals were more active than their SEC counterparts, this finding was more pronounced in the postpartum animals. Furthermore, when comparing the postpartum SEC and IC animals with their SC counterparts, these activity differences appear to be due to reduced SEC levels of performance whereas in the virgin animals, comparison of the SEC and IC groups with their SC conspecifics, suggests that heightened IC activity levels contribute most to the SEC/IC differences. Additionally, in the virgin groups, SEC females reared the most (compared with both the IC and SC groups), suggesting that these animals were the most exploratory, the opposite pattern emerging in the postpartum groups.

Finally, if time in centre is a measure of emotionality, then from the evidence, few differences in emotionality exist in the virgin animals. By comparison, however, in the postpartum groups SEC animals were more emotional than their IC counterparts.

So in response to the question "do environmental effects continue postpartum?", the patterns of behaviour which emerged demonstrate that although there are significant differences between SEC and SC/IC animals postpartum, justifying the use of these environments as a maternal manipulation, it should be noted that the postpartum animals' performances were qualitatively different from their virgin counterparts. In particular, in the latter groups, impoverishment produced animals with higher levels of activity, enrichment enhancing exploratory and perceptual skills. In the postpartum groups, however, enrichment reduced activity and exploration whilst increasing emotionality, relative to animals exposed to impoverishment prior to pregnancy. This suggests that the consequences of pregnancy and parturition have the greatest impact on the behaviour of the enriched animal.

9:2:2 BEHAVIOURAL PROFILE OF OFFSPRING AND GRAND-OFFSPRING GROUPS

The second aim of this thesis was to establish a behavioural profile of the offspring and grand-offspring of differentially housed mothers, that is to investigate the intergenerational transfer of environmental effects. Study two (chapter six) was therefore designed to investigate whether the effects of exposing females to SEC, SC and IC prior to pregnancy altered the behaviour of their offspring (experiment one) and grandoffspring (experiment two). In this discussion chapter, these profiles will be briefly summarised and the pattern of behavioural differences over generations highlighted.

a) Behavioural Profile of Offspring

In order to establish a behavioural profile of the offspring groups, animals were examined in similar test situations to those employed in the previous study, with their parents' generation. Results indicated that manipulating the environment of a female rat can significantly influence her offspring.

Considering first offspring open field data, on day one both SC and IC offspring crossed significantly more lines than their SEC counterparts, but did not differ significantly from each other. If the SC offspring are seen as the control group, this suggests that the performance differences between the groups (on day one) are due to reduced reactivity in the SEC offspring compared with their IC conspecifics. Offspring groups' patterns of responding over days also varied, both SEC and IC offspring groups tending to increase their activity patterns over days (comparing their day one with day five performances), SC groups, although maintaining higher levels of activity than the SEC and IC progeny groups on the first four days of testing, tending to reduce their relative pattern of activity as measured by number of lines crossed, over the last four days of testing.

With the rearing measure, however, although the tendency was for both the SEC and IC groups to increase their numbers of rears over the five days of testing (reminiscent of their lines crossed behaviour) with the SC progeny maintaining a similar pattern across days, the relative position of the three groups were very different from their lines crossed performances. In this instance, over days IC progeny reared more than their SEC and SC counterparts. This suggests both that these animals are more exploratory and that it is the impact of maternal impoverishment rather than enrichment which is contributing to this effect. When the time in centre measure is taken into account, again the effects of maternal impoverishment were noted, differences emerging between IC offspring and their SEC and SC counterparts, offspring of IC dams being the most emotional.

With the visual cliff, no significant differences emerged between the three offspring groups on

the side chosen measure, not a very surprising result given the similarity of direct experience of depth these animals had had. However, a significant interaction in the latency to descend measure between the three offspring groups' performances on the two cliff depths did emerge. When the cliff depth was set at one inch, both the SEC and IC offspring groups took less time to descend from the central platform compared with their performance when the cliff was set at twelve inches, the opposite pattern emerging for the SC offspring. Although this may initially suggest that the SEC and IC offspring groups were responding to the depth of the cliff, the lack of significant differences in side chosen measures does not appear to support this hypothesis. Finally, offspring performances were examined in a Skinner box situation. In this instance, higher bar press rates were noted in the SC progeny, when compared with their SEC counterparts, no other group differences emerging.

To summarise, therefore, when comparing the offspring of SEC and IC dams with their SC counterparts, maternal enrichment appears to contribute most to the day one open field activity differences and may have a role to play in bar press responses. Maternal impoverishment on the other hand, appears to contribute most to the group differences in exploratory behaviours and emotionality. Why these differences exist, however, is less obvious.

Throughout the discussion of chapter six, offspring profiles were compared with those of their parent generations. As this discussion progressed it became clear that although significant differences had emerged between the three offspring groups behavioural responses in the various test situations, the patterns of differences in these animals were very different from the pattern of differences observed between their parent generation, animals exposed directly to SEC, SC and IC. Furthermore, the offspring groups performances were neither consistent within nor across test situations.

However, consideration of the EC/IC literature coupled with an examination of those few studies in which exposure to differential environments have been employed as a maternal manipulation did allow certain possible causal mechanisms to be highlighted. In particular, it has been sug-

gested that biochemical, endocrinological and arousal differences between the maternal groups (Ivinskis and Homewood 1980; Kiyono et al 1985; Diamond 1987) might be contributing to the offspring differences, as might differential maternal stress from their qualitatively different environmental experiences. As no biochemical or endocrinological assays were taken in this study, no comments could be made about offspring differences with respect to these measures, other than to state that it is possible that changes at these levels of analysis would accompany the offspring behavioural differences (cf Kiyono et al 1985). However, given that performances differences have been found to be related to stress (chapter three) and arousal (Yerkes and Dodson 1908) and that this study had observed behavioural measures often associated with stress and arousal (for example activity and emotionality), the hypothesis that offspring behavioural differences might be subserved by differential stress and/or arousal levels was examined further. Although neither stress nor arousal were able to account for the complete range of offspring behaviours, some aspects of the results could be interpreted within these frameworks. In addition, the possibility that offspring groups' performance might reflect differential learning ability was also considered and a further set of experiments suggested to tease out the relative contribution of these postulated mechanisms.

Prior to describing the results of these experiments, however, and assessing their contribution to an understanding of the offspring groups' performances, the final aim of this chapter, the establishment of behavioural effects over two generations needs to be considered.

b) Behavioural Profile of Grandoffspring

The behavioural profiles of the three grandoffspring groups were examined in the second experiment of study two (chapter six). Unlike the previous generation, grandoffspring of SEC dams were significantly more exploratory than grandoffspring of either SC or IC dams, as measured by the number of rears they made in the open field. In addition, although not statistically significant, both SEC and SC grandprogeny groups tended to be both more active and to bar press

more than their IC counterparts. These results suggest, that as with the previous generations, the relative contributions of enrichment and impoverishment may vary according to behavioural measure. For a more complete picture of these grandoffspring differences, however, they need to placed in the context of the intergenerational differences observed in the three generations studies in this thesis. This will be undertaken in the following section.

c) Comparison of Profiles over Generations

When the profiles of the SEC, SC and IC animals, their offspring and grandoffspring are compared, two striking facts emerge. Firstly, as the effects are passed across generations, the relative patterns of behaviour between the groups change, such that the contributions of maternal enrichment and impoverishment have a differential impact on subsequent generations. Secondly and not unusually, as the impact of differential maternal environments are filtered across generations with no additional "topping up" of environmental experience, the behavioural differences between the groups become less statistically significant.

In particular, in animals exposed directly to differential environments, the activity levels of IC animals remained high across days, as measured by numbers of lines crossed, when compared with their SEC and SC counterparts (Figure 5:1), suggesting that the differential effects were due to impoverishment raising animals' activity levels. Postpartum, however, both IC and SC dams were more active than their SEC counterparts (Figure 5:6), suggesting that in this instance, activity differences were due to the effects of enrichment. In the offspring generation, although the tendency was for SC progeny to decrease their activity over days, SEC and IC progeny increasing their activity levels, SC offspring tended to be more active over the first four days of testing than either their SEC and IC conspecifics (Figure 6:1). With the grandoffspring generation (Figure 6:5) although not statistically different from each other, both SEC and SC grandprogeny tended to be more active than their IC counterparts, again highlighting impoverishment rather than enrichment.

With exploration, as measured by number of rears in the open field, again different patterns emerged across the generations. In animals exposed directly to differential environments, SEC animals reared more than either their SC or IC counterparts (Figure 5:3), a result which has been attributed to the effects of enrichment. In the postpartum groups, however, SEC animals were less exploratory than their SC and IC littermates (Figure 5:7). Interestingly, by the time the effects of differential environments had passed across one generation, maternal impoverishment appeared to be contributing most to offspring group differences. As can be seen from Figure 6:2, IC progeny reared more than their SC and SEC counterparts. Across two generations, however, the most exploratory animals were the grandoffspring of enriched rats (Figure 6:6), grandmaternal enrichment appearing to have contributed most to the differences observed between the three grandoffspring groups, reminiscent of the effects noted in animals exposed directly to differential environments and tested immediately after their environmental experiences.

When considering group differences in emotionality, some confusion appears in the data. In one of the most commonly employed measures of this construct, number of defecations in the open field (Whimbey and Denenberg 1967b), no differences were reported between the three experimental groups in any of the generations. If time spent in the centre circle is considered with animals spending less time in the centre being seen as more emotional, however, differences were found between the experimental groups, both in the postpartum animals and in the offspring generation. In particular, SEC animals tested subsequent to the weaning of their litters were found to spend less time in the centre circle than their IC counterparts [4], in the offspring generation, the opposite pattern emerged. In this instance, IC animals spent less time in the centre circle than either their SEC or SC conspecifics, suggesting that maternal impoverishment rather than enrichment contributes most to offspring emotionality differences. Why there is a lack of consistency between these measures of emotionality is, at present, unclear. Either one or other of these behavioural measures does not tap emotionality, or, as has become increasingly obvious from the experiments in chapter seven and eight, there is a lack of consistency in animals' performances within and

[4] The relative contributions of enrichment and impoverishment to this result, however, cannot be ascertained, as the SC group do not differ significantly from either the SEC animals or their IC littermates.

across tasks.

Finally, Skinner box behaviours were also measured in the three generations. In animals directly exposed to the differential environments, IC animals bar pressed significantly more than their SEC counterparts (Figure 5:5). When compared with their SC counterparts, however, gender differences emerged. In particular, enrichment appeared to contribute most to the bar press differences in males, impoverishment having the most impact in females. When considering the offspring performances under the same reinforcement levels (chapter six), enrichment appeared to contribute most to the effects (Figure 6:4). In the grandoffspring generation, however, although not statistically significant, if anything grandmaternal impoverishment had more of an impact across generations in this measure (Figure 6:7) than grandmaternal enrichment, grandprogeny of SEC and SC animals tending to bar press more than their IC counterparts.

One obvious question to arise from these results is why are the successive generations behaviourally different? One possible answer concerns the *timing* of the manipulations. In animals directly exposed to differential environments, the environmental experiences they are subjected to typically occur postweaning. In the offspring and grandoffspring generations employed in this thesis, however, the experimental manipulations were present at an earlier stage in their development. The impact of qualitatively different levels of stimulation provided by interactions with the maternal generation in both the offspring and grandoffspring generations occured from birth to weaning, a time when the developing organism is particularly sensitive to its environment (Thompson and Grusec 1970). Furthermore, although not tested directly in this thesis, offspring of differentially housed mothers may also have been influenced by their dams in utero, adding to the nature of their experience. Indeed, as noted in chapter three, in the prenatal stress literature, both the *timing* and *nature* of the maternal manipulation have profound impacts on offspring generations. It is not unreasonable to assume that differential environments as a maternal manipulation also follow this pattern.

Before addressing the possible causes of these differences, however, to obtain the full behavioural

picture of the offspring groups, the final two studies of this thesis need to be considered.

9:2:3 FURTHER ANALYSIS OF OFFSPRING PERFORMANCES

In study two offspring of differentially housed mothers were found to differ, both in terms of their learning performance and their open field activity. In the discussion of the study (chapter six) it was suggested that a variety of factors may have influenced offspring behaviour, including:

- Firstly, that offspring groups, having experienced qualitatively different mothers (Muir et al 1985), might have been differently stimulated and thus might differ in terms of baseline arousal levels (Walsh and Cummins 1975; Ivinskis and Homewood 1980).
- Secondly, given that the three maternal environments might be seen as differentially stressful (Uphouse 1980), it was suggested that other intervening variables such as endocrine or neurochemical mediators may well be partly responsible for the effects, such as would be predicted by both the enrichment literature (Renner and Rosenzweig 1987) and the prenatal stress literature (chapter three).
- Thirdly, offspring effects, it was suggested, might simply reflect the differential opportunities for learning afforded by exposure to mothers with fundamentally different behavioural profiles.

In order to explore these causal hypotheses further, the final aim of this thesis was to provide a more detailed analysis of offspring performances in both learning and activity based tasks. This provided the impetus for designing the last two studies of this thesis (chapters seven and eight) the more specific objectives of which are considered in the following subsections.

a) Study Three (Chapter Seven)

The purpose of this chapter was twofold: Firstly, to explore further the mechanisms which might explain the observed offspring behavioural differences, concentrating in particular on the possibility that differences in offspring performances might reflect differential learning capacities, stress and/or arousal levels. Secondly, within the EC/IC literature itself, one source of interest in the effects of differential environments which stems from its inception (Hebb 1947) is the notion that enrichment produces an animal which is more "intelligent" than its impoverished littermate. In the present thesis, the possibility that the beneficial effects of enrichment could be passed across generations was of considerable theoretical importance. Consequently, this study had an additional impetus, namely to investigate whether or not offspring of enriched animals did have increased cognitive abilities.

In experiment one, reasoning that if offspring of SEC, SC and IC dams do have different learning capacities then these should be most obvious in tasks which test directly for learning, a Hebb-Williams maze paradigm was employed. In experiment two, a Skinner box procedure developed by Rose et al (1986) was used, which they suggested distinguished experimentally between EC/IC differences in learning capacity per se and differences in motivation. Results of these two experiments will be summarised below.

HEBB-WILLIAMS PERFORMANCE

Offspring of SEC, SC and IC dams were put through a training and testing procedure based on that employed by Rabinovitch and Rosvold (1951), during which time behavioural measures of activity, learning and emotionality were taken. Most of the differences between the offspring groups emerged in the dependant variables measuring activity and emotionality. In particular, in the pre-training phase, SC offspring took longer to eat in the goal box than their SEC counterparts whilst in the training maze (day one) both IC and SC progeny took longer to reach the goal than

the SEC progeny, highlighting the role of maternal enrichment in these effects. Typically, IC offspring took longer in the initial trials in the test mazes, both with respect to latency to emerge, time to reach the goal box and total time, suggesting that these animals were more emotional. IC progeny were also more active in the early mazes, their SEC counterparts being more active in the later ones. The only hint of a learning difference between the three offspring groups emerged in the early phase of training, SEC offspring taking less time to reach criterion than the SC group, these animals in turn taking less days than their IC counterparts.

If, however, this last result is examined more closely and the requirements for reaching criterion examined (namely traversing the practice maze nine times in less than one minute on two consecutive days) factors such as emotionality and activity were found to contribute more to this effect than learning capacity per se.

Overall, these results were taken to indicate that although exposing female animals to differential environments do produce qualitatively different offspring, it is unlikely that these differences reflect altered cognitive capacities. Indeed, differences in activity and emotionality rather than learning best described their profiles and from the patterns of differences which emerged, it was suggested that the offspring of differentially housed mothers might be either be differentially stressed and/or aroused. When these mediating mechanisms were explored further, as with the previous study (chapter six), neither proffered a complete explanation of the results. Indeed, given the lack of consistent findings across various test situations, it was suggested that maybe both stress and arousal (with their accompanying changes in biochemistry at neuronal and endocrine levels) were interacting to produce the results. It was decided therefore to test this further, by manipulating one of these hypothesised mechanisms and for theoretical and practical reasons offspring arousal levels were chosen.

OPERANT CONDITIONING TASK

In this experiment, reasoning that if offspring groups' behaviours were in any way being influenced by differential arousal levels, then by externally mediating their arousal levels, through the use of a highly stimulating reinforcer in a Skinner box paradigm, certain behavioural predictions could be made. In particular, in an earlier experiment (study two), SC offspring were found to bar press significantly more than their SEC counterparts. IC progeny, although not differing statistically from either the SEC or SC groups, produced bar press levels which fell between their SC and SEC conspecifics. These performances, it was suggested, might reflect differing positions on an inverted-U arousal-performance curve (Yerkes and Dodson 1908; Hebb 1955), such that SC animals being optimally aroused, produced optimal performance levels, the lower performances of the other two groups reflecting either higher or lower arousal levels. In this experiment, by employing a more stimulating reinforcer, it was predicted that all three offspring groups' arousal levels would be increased, with a resultant shift along the hypothesised arousal-performance curve, such that the rank ordered position of the offspring groups, in terms of bar press rates, would also change.

Results demonstrated significant differences between the three groups and in part, supported the hypothesis that offspring groups were differentially aroused. For example, SEC offspring, whose previous Skinner box performance was significantly lower than their SC counterparts, increased their bar press rates as would be predicted by an arousal hypothesis. In addition, these animals were significantly different from the IC progeny, the latter animals having lower bar press rates. However, the SC group's performance was similar to that of the SEC group, not corresponding exactly to the experimental hypothesis [5]. Prior to drawing any firm conclusions about these results, however, one final experimental procedure was suggested, to test the arousal hypothesis to its limit. This provided the focus of the last experimental study, chapter eight.

[5] In this test situation the main impact of differential maternal environments appears to be due to maternal impoverishment, unlike the previous Skinner box study in which if anything, maternal enrichment was having the main effect. NB: the differences between these studies lies in the nature of the reinforcer, obviously interacting differentially with the maternal conditions.

b) Study Four (Chapter Eight)

This study was designed quite specifically to test the hypothesis that offspring of differentially housed mothers might have different underlying baseline arousal levels. Two behavioural procedures that had demonstrated clear differences between the offspring groups in study two (the open field) and study three (the operant conditioning task) were employed and animals' arousal levels chemically manipulated with *d*-amphetamine sulphate, as recommended by Walsh and Cummins (1975).

Several hypotheses relating to specific behavioural patterns expected in the offspring under the various drug regimes and test procedures were advanced, including:

- In the Skinner box paradigm, pre-drug bar press rates over the last test days prior to drug administration will differentiate between the three offspring groups, such that SEC and SC progeny will bar press more than the IC progeny.
- In the saline condition, where animals are injected but not administered any amphetamine, SEC and SC offspring will continue to bar press more than their IC counterparts.
- As the dose levels of amphetamine increases, all groups' performances will increase initially and then decrease, lowest levels of performance being found with the highest doses.
- The dose-response curves for the three offspring groups will differ such that the IC offspring will reach an asymptotic level at a lower dose than their SC and SEC counterparts. This will be represented by a group by dose by days interaction.
- In the open field, it is predicted that the three offspring groups will also differ in their dose-response curves, SEC animals reducing their levels of activity as measured by lines crossed and rearing behaviours at higher doses than the other two offspring groups.
- Animals receiving saline will have open field behavioural profiles that are similar to those reported in chapter six (study two).

In this study, the results failed to find any statistically significant differences between the offspring groups' dose-response curves, suggesting that the observed group differences noted in previous experiments cannot be described in terms of a unitary intervening variable such as arousal. However, when the results of the two experiments are considered, several interesting points did emerge.

Considering the Skinner box data first, unlike the experiment reported in chapter seven, no differences between the offspring bar press rates prior to drug administration emerged. This, it was suggested initially, might reflect the pre-asymptotic performance levels of the groups and it was further suggested that offspring differences might have occured if the training period had been maintained for longer. However, when this hypothesis was explored further, both by considering the saline group and by comparing subjects responses over the pre-drug training days with those of the previous Skinner box study (chapter seven), it was found to lack empirical support. In particular, no differences emerged between the three offspring groups over the four days of testing under the saline condition, although there was a tendency for the IC offspring to bar press more than their SEC counterparts in the four drug dose conditions (see Figure 8:2). Furthermore, in this study, no pre-drug diferences were found between the three offspring groups, different learning patterns having emerged in the earlier study over the equivalent training period.

These findings are in direct contradiction to those predicted by the arousal hypothesis. However, as will be discussed in more detail in sections 9:3 and 9:4, it should be mentioned at this point that the procedure employed in this study, namely injecting the animals, is in itself stressful and may well have altered their arousal levels. Consequently, these results should be treated with some caution. Finally, as was predicted, all offspring performances were affected by the dose of drug, such that the lower doses increased the performances of all the groups, higher doses reducing performances.

With the open field, considering the dose-response curves first, although there were no significant differences between the groups' patterns of responding over days for either the number of lines

crossed or rears measures, as can be seen from Figure 8:4, SEC and SC offspring tended to be more active than their IC counterparts at the lower doses. That is, these animals' responses were in the predicted direction. However, at the higher dose levels these animals were still highly active rather than reducing their levels of performance. It was suggested that these animals had not reached their ceiling levels of performance.

Overall, these results do not appear to support the hypothesis that offspring of SEC, SC and IC dams are differentially aroused, or at least indicate that the notion that arousal is *the* mechanism subserving the changes observed over generations is too simplistic. The obvious question is therefore, what factor(s) are involved in these intergenerational effects? Before this question can be addressed, however, there are certain methodological issues that must be considered, as they may impinge upon the interpretation of the findings. These methodological issues will be considered in the next section, followed by an overview of the possible causes of the intergenerational effects.

9:3 METHODOLOGICAL CONSIDERATIONS

In this thesis, certain methodological decisions were made in the design of the four studies, which may influence the interpretation of the results and as such should be highlighted. It should be emphasised that all of these decisions have been discussed already in the relevant experimental chapters. However, they are worth reiterating here, in the context of an assessment of the present research. For ease of presentation, an evaluation of the design of the studies will be undertaken by successive chapters but it should be noted that some design decisions have permeated across chapters and therefore, must be seen in a more global context.

a) Appraisal of Chapter Five

In evaluating the superenriched environment employed in this thesis, male and female SEC animals were compared with their SC and IC counterparts. An alternative method of evaluation

would have been to compare animals housed in the SEC with those housed in the more traditional type of enriched environment typically employed by Rosenzweig and his colleagues, the EC. However, as the EC had been extensively reviewed in chapter two, this was considered a sufficient literature against which to compare the SEC animals' performances. Furthermore, certain practical considerations had to be taken into account in this study, namely amount of colony room space available to the present author and number of animals that could be successfully tested by one experimenter. In chapter five, experiment one, some sixty animals were tested in a variety of behavioural meaures that are by their nature time consuming. Including two enriched environments (EC), one for male and one for female animals in the design of this experiment would have increased the number of animals to eighty, just too many to easily test in the open field and visual cliff procedures. Staggering animals' housing and testing was avoided wherever possible, to ensure that conditions were similar between experimental groups, consequently in this thesis it was decided to compare SEC animals with their SC and IC counterparts, rather than include an additional EC environment.

Secondly, in this study, all animals were individually housed for one week, prior to testing. Technically, therefore, all animals were tested from isolation. However, as pointed out in the procedure section of this chapter, this delay in testing and housing arrangement has been employed elsewhere in the literature (Rose et al 1985; 1986; Dell and Rose 1987) and does not appear to alter the typical EC-IC effects reported in chapter two (Lamden 1985). In future research, however, given that the effects of isolation can have an impact on brain measures in a very short space of time (see chapter one), it is recommended that animals should be maintained in their respective environments throughout the testing period.

A third design factor which was taken into account, was the order of testing in experiment one of this chapter. In particular, animals were tested in the open field first, then the Skinner box and finally the visual cliff. This order was determined by the relative contribution of one test to ensuing ones. The open field relies on tapping an animal's response to a novel environment and of

the three tests used is the one which benefits most from naive animals. Consequently it was the first test to be employed. As training and testing animals have been found to have an "enriching effect" (Rosenzweig and Bennett 1977), of the two remaining tests, it was felt that the visual cliff, which tests perceptual ability, would be least influenced by the impact of testing experience, consequently this procedure was carried out last. In this experiment, results were similar to those noted in previous studies and so it is likely that test order did not significantly influence animals' responses. However, in an ideal design, naive animals would have been employed for each of these procedures, tripling the number of animals required.

A fourth element to be considered in this study is its sample size. This had an impact on the analysis of the visual cliff data, the relatively small number of animals in each cell (N=20) [6] reducing the chance of attaining a significant result. As noted, above, the sample size was determined by practical considerations. However, if this experiment were to be replicated, the author would advocate employing a larger sample to ensure that expected frequencies did not fall below five in any one cell.

Moving on to the second experiment in this chapter, in which postpartum effects were investigated, only one behavioural measure was employed, the open field. As in this experiment, unlike the previous one, no attempt was made to provide a behavioural profile of these animals it was deemed sufficient to test for behavioural differences in one experimental procedure. The choice of the open field reflected its' importance in the literature as one piece of apparatus which does distinguish EC and IC animals' behaviour. However, one obvious criticism of this experiment is that in concluding that effects continue postpartum, it must be noted that this is only true for the open field and one should be aware of the problems of generalising this finding to other test procedures. At the time, this design decision seemed reasonable to the present author. However, in the "ideal" experiment, postpartum groups should be tested on a wider range of apparatus.

Finally, in this experiment, no attempt was made to measure brain changes in the postpartum

[6] The sample size is seen as small, when employing a chi-square analysis. In most experiments reported in the EC/IC literature, however, this sample size is the norm.

groups. This was due to the lack of laboratory facilities at the time of testing. In future, however, it is recommended that neuroanatomical measurements and biochemical assays be standard practice in this type of research.

b) Appraisal of Chapter Six

In this study, the behavioural profiles of offspring and grandoffspring of SEC, IC and SC dams were investigated. As with the previous chapter certain methodological decisions were taken which were compromises based on the best possible design given certain practical constraints. In particular, in the first experiment in this study, offspring of differentially housed mothers were each tested in two pieces of apparatus. As with the previous study, the open field apparatus was employed first, then half the subjects were tested in the Skinner box apparatus, half in the visual cliff. This was an attempt to take into account the problems of training itself influencing test results (Rosenzweig and Bennett 1977) by reducing the number of experiences of the groups, whilst still keeping numbers of subjects as large as was possible given the breeding constraints.

The practicalities imposed by the space available in the colony room for the breeding programme also influenced the second experiment in this chapter, where grandoffspring were investigated. In particular, the complexity of the breeding programme and the number of animals involved to produce the experimental sample resulted in a decision being made to restrict the experiment to thirty offspring and more specifically thirty male offspring. This latter decison was based on two factors. Firstly, in much of the EC/IC literature, effects have been investigated in male animals. If one sex of animal had to be chosen in this experiment, the precedent established in previous work dictated that it should be male animals. Secondly, at this time, only preliminary data analyses had been performed on the offspring data, to ascertain if effects existed between animals of mothers raised in SEC, SC and IC, before moving on to consider effects over two generations. This limited analysis was a deliberate decision, to reduce experimenter effects (Rosenthall 1966). However, when complete analyses were performed, sex differences between the groups were noted.

As mentioned in the introduction to this experiment, with hindsight, it would have been preferable to include female offspring too.

c) Appraisal of Chapter Seven

As with the previous study, only male offspring were employed in the Hebb-Williams and operant conditioning tests in this study. As noted in the introduction to this work, the choice of male animals reflected the precedents set by previous literatures (Kiyono et al 1985; Dell and Rose 1986) as well as the limits imposed upon sample sizes by the amount of space available in the colony room for the breeding programme. Given that research is often compromised by practical considerations, it is the present author's opinion that the experimental decisons made are entirely justifiable. However, future research, wherever possible should endeavour to include both male and female animals.

d) Appraisal of Chapter Eight

Of the four studies in this thesis, the study presented in chapter eight (study four) is the one which presented the author with the most difficult decisions. As noted in the introduction to the experiments, several methodological questions had to be addressed, including choice of behavioural test, test order, drug dosage and administration and types of dependant variables measured. The choices made were all justifiable, both in terms of precedents set in the literature and theoretical relevance to the thesis itself. However, some additional factors should have been taken into account, which only emerged as problematic once the data had been collected.

Firstly, in the present thesis an independent subjects design was employed, animals only being given one dose level of the drug *d*-amphetamine sulphate. As Will and Checchinato (1973) have noted, however, there is a large inter-subject variability in bar press responding to amphetamines and an alternative between-subjects design may have been more appropriate in the Skinner box

experiment. Furthermore, subjects were only given three days of treatment with amphetamines in this operant conditioning task. Perhaps for the effects of amphetamines to become clear, a longer period of administration would have been necessary. The limiting of the number of days of drug administration was because in this study the subjects were young and still growing rapidly. As amphetamines have been found to interfere with appetite and may have influenced normal growth patterns in the subjects, care was taken to administer as little of the drug as was possible. Perhaps, in retrospect, the author was too cautious.

Probably the most problematic aspect of the present research, however, concerned the choice of appropriate control group. In this study, baseline levels of performance were taken from animals of dams exposed to SEC, SC and IC, which had been injected with saline. However, it is quite likely that this experimental procedure was stressful and as Curry (1987) has noted, may well have altered these animals' arousal levels. A more appropriate control might have been to employ sham injections [7], such as were used by Curry (1987) so as to reduce the arousing properties of the control procedure. Given that the control groups may have been inadvertantly aroused by the procedure employed in this study, it is the author's opinion that the results of this experiment must be considered with some degree of caution.

Overall, despite the methodological problems outlined in more detail above, it should be remembered that clear differences were found between the offspring and grandoffspring of female rats exposed to differential environments prior to pregnancy. As one of the aims of this thesis was to begin to unravel the nature of these differences, in the next section an overview of possible causes of these effects will be discussed.

[7] This involves holding the animal in a manner similar to that of animals to be injected, but holding an empty syringe with no needle against the animal. This procedure simulates the effects of handling, but does not allow the animal to be invaded by a needle, nor does it include any introduction of saline into the animal's system.

9:4 POSSIBLE CAUSES OF THE INTERGENERATIONAL EFFECTS

Throughout this discussion, offspring and grandoffspring groups' performances have been considered in a range of behavioural tasks. Two important facts have emerged. Firstly, it is now clear that the effects of differential maternal environments across generations are complex, there being a lack of consistency in offspring groups' behaviours both within and across test situations. Secondly, the relative contribution of maternal enrichment when compared with impoverishment also varies, both within and across tasks and across generations.

As has become increasingly apparent, unravelling the causes of these effects is problematic, both because the results are difficult to interpret and because they may reflect a range of underlying mechanisms. Consideration of those few studies in which differential environment have been employed as the maternal manipulation (eg: McKim and Thompson 1975; Ivinskis and Homewood 1980; Kiyono et al 1985; Diamond 1987) coupled with the variety of factors which have been forwarded to account for the effects of *direct* exposure to differential environments and which may indirectly contribute to the observed differences in offspring and grandoffspring generations, however, does offer a starting point. From these literatures certain possible causes were highlighted in chapter six, including endocrine system alteration, neurochemical changes, learning, stress and differential arousal. In the light of the experimental studies reported in chapters seven and eight, however, these causal hypotheses need to be reassessed.

Considering first endocrine system alterations and neurochemical changes, as no biochemical assays were taken in this thesis, little can be said with respect to these measures at this juncture. However, as will become apparent in this discussion, in the author's opinion, it is at this level of analysis that future research should be directed. Prior to exploring this further, however, mechanisms which were amenable to behavioural research and which were investigated further in chapters seven and eight will be dealt with first.

Probably one of the most clear cut findings of this thesis, and arguably of the greatest theoretical

interest, is that whatever is being transferred across generations it is not having an impact on the offspring groups' problem solving ability (see chapter seven). Furthermore, although group differences in Skinner box performance were observed in chapter six, the manipulation of the nature of the reinforcer in the second Skinner box study (chapter seven) with its resultant changes in the relative patterns of offspring bar press rates when compared with those observed in chapter six, suggests that any performance differences observed in this apparatus were being subserved by mechanisms other than learning ability. Indeed, given that the only differences to emerge in the Hebb-Williams study were in terms of offspring emotionality and activity, it now seems more likely that it is differences in these types of behviour which best describe the offspring performances in all of the learning test situations.

Obviously the lack of differences between the offspring groups in learning capacity does not mean that the SEC and IC are not producing changes in learning and memory in the maternal generation. Indeed, there is now considerable evidence that one of the differences between these types of environments is the differential opportunity for learning afforded their incumbents (Greenough 1976; Renner and Rosenzweig 1987). What is apparent from the present results, however, is that any beneficial changes in cognitive capacity in the maternal generations are not passing across to the offspring and grandoffspring generations in some "non-genetic" manner. Furthermore, it now seems unlikely that the differential mothering behaviours noted by Muir et al (1985) are affording the offspring groups qualitatively different learning opportunities. Indeed, if there are any effects of the maternal generations' differences in learning on their offspring at all, it is more likely that these effects are subtle, possibly emerging in the mothers' differing levels of adaption to novelty, with resultant alterations in stress levels and hormones, the latter having an impact on the offspring groups' activity and emotionality. Obviously this is, at present, an untested hypothesis, warranting further research.

Differences in offspring groups' activity and emotionality, however, such as have been observed in several of the test situations employed in this thesis, can be explained by factors other than

maternal ease of adaption to pregnancy, parturition and litter-rearing. Firstly, the maternal environments might themselves be seen as stressors, in the same way as immobilisation, crowding or handling of the maternal generation, for example, have been considered as stressors (chapter three). These differential environments (with their postulated varying degrees of stress for their incumbents) may in turn, have an impact on the offspring either in utero or via the maternal-infant interaction postpartum (Denenberg and Whimbey 1963). Alteratively, differences in offspring levels of emotionality and activity may simply reflect differences in their baseline arousal levels perhaps being mediated by qualitatively differing levels of stimulation afforded by the different types of mothers they had experienced (Walsh and Cummins 1975; Ivinskis and Homewood 1980; Muir et al 1985).

Considering the notion that the offspring behavioural differences observed in this thesis are a product of differential maternal stress, two criteria need to be satisfied. Firstly that the differential environments can themselves be considered differentially stressful and secondly, that the offspring groups' patterns of behaviour are consistent with a stress hypothesis [8]. With respect to the idea that the SEC, SC and IC are differentially stressful, there is still considerable debate within the literature (Rosenzweig and Bennett 1976; 1978; Uphouse 1980; Renner and Rosenzweig 1987) as to whether or not EC-IC differences can in any way be attributed to stress. In particular, it has been suggested that the enriched environment may be stressful, in that it overstimulates its incumbents resulting in "information overload", whilst the IC provides a form of sensory deprivation resulting in "isolation-induced stress" (Uphouse 1980 p225). However, the two main approaches that have been used to assess the contribution of stress to EC-IC differences, measurements of physiological indices of stress and comparison of EC and IC animals with animals subjected to other forms of stress, have provided conflicting results.

More specifically, although there are problems in defining the concept of stress and its concomitant physiological indices (Uphouse 1980), the most commonly employed physiological measures,

[8] That is, parallel results observed in offspring of mothers that have been stressed by manipulations other than exposure to differential environments.

increased adrenal weight and/or adrenal response (increased output of ACTH or adrenal corticosteroids), have produced mixed results. Although adrenals have been found to be enlarged in impoverished animals (Geller, Yuwiler and Zolman 1965; Uphouse and Bonner, unpublished data cited in Uphouse 1980) not all of the studies have found this difference to be significant (Greenough 1969; Krech et al 1966). Uphouse (1980) has pointed out, however, "that the presence or absence of enlarged adrenals may depend on the particular strain (of animal) investigated. Alternatively, the adrenal response may not be permanent or may depend on other rearing variables that differ across laboratories." (p226), making this type of investigation methodologically problematic. Furthermore, the other commonly accepted physiological index of stress, adrenocortico activity, has only been measured once (Geller 1971) and has provided little evidence of long term stress responses in the EC-IC animals. In this study increased adrenal corticoid output was noted in the impoverished animals during the first part of their exposure to the environment, but by the eleventh day, no differences were found between the EC and IC groups. Geller suggested that the animals had quickly adapted to their respective environments and were no longer responding by adrenal activation. That is, if there were any stress effects, they were of short duration. If this were the case, however, then as Uphouse (1980) has succinctly commented, the presence of this "transient adrenal response should lead us to exercise caution in restricting measurements to the conclusion of the rearing interval and interpolating to the events that preceded it" (p227). That is, any measurement of adrenal weight should occur earlier in the procedure, rendering the results presented above, methodologically unsound.

Physiological measurements are not the only experimental evidence which has been forwarded as evidence against the role of stress in the EC-IC effects. Riege and Morimoto (1970) compared animals exposed to EC and IC with groups that had also been exposed to a daily 'tumbling' stress, predicting that in the stressed groups, the EC-IC differences should disappear as the EC group would have also been subjected to environmental stress. This did not occur, leading these researchers to conclude that whatever the causes of the EC-IC differences, they could not be due to stress. However, this type of analysis presumes that the effects of tumbling would be similar

(or equivalent) to the effects of isolation, or that the effects of the two types of stress (isolation and tumbling) are not cumulative. As Uphouse has noted "in general it is correct to conclude that the effects of differential rearing are not mimicked by the conditions of daily tumbling. However, to conclude from these data that "stress" is not influential in EC-IC differences is premature" (1980 p228).

From the above studies, one can either conclude that there is little firm evidence that stress is causing the EC-IC differences, a position advocated by Renner and Rosenzweig (1987), or more reasonably that the evidence is inappropriate (either methodologically or theoretically) and the impact of stress on the animals exposed to differential environments remains to be illucidated. Either way, for the purpose of this thesis, there is little evidence to date which conclusively proves that exposure to differential environments is differentially stressful. On a more positive note, however, there is no reason to suppose from the evidence presented above, that stress is not involved. Indeed, intuitively one might well consider the effects of isolation to be stressful, whether or not the animals adapt to their circumstances over time, a fact which may well have an impact across generations if not having a permanent effect on the maternal generation itself.

The second condition which needs to be met before the behavioural differences noted in this thesis can be attributed to differential maternal stress, is whether the patterns of offspring behaviour are consistent with a stress hypothesis. As has become apparent in this thesis (see chapter three), both the timing and the nature of the stressor plays an important role in determining its effects on the offspring (and grandoffspring) generations. Consequently to expect any one particular effect of stress is inappropriate. What is to be expected, however, is that the effects of maternal enrichment and/or impoverishment should be consistent both within and across tasks. As can be seen from the offspring profiles which have emerged in this discussion, the relative contributions of maternal enrichment and impoverishment to various behavioural measures have not been consistent. For example, in the Skinner box experiment in chapter six, maternal enrichment contributed most to the SEC and IC offspring groups' performances, in chapter seven,

offspring bar press rate differences were most influenced by maternal impoverishment. Similar variations in the relative contribution of maternal enrichment and impoverishment to offspring group differences in emotionality across test situations can also be identified [9]. It is this lack of consistency which suggests that whatever is causing the offspring differences, it cannot simply be different types of maternal stress. That is not to say that stress is not having an impact. Indeed, given some of the similarities between the present findings and those reported in chapter three, with effects appearing in measures of emotionality and activity rather than learning, it is difficult to dismiss stress altogether as a causal mechanism. What must be concluded at this juncture, however, is that stress is not the main causal mechanism and other levels of analysis must now be entertained.

Moving on to an alternative explanation, the possibility that offspring groups differ in terms of their baseline arousal levels, initially the results of the last study undertaken in this research appear to cast doubts over the notion that offspring of SEC, SC and IC dams are differentially aroused. However, when the methodological problems associated with this study are taken into account, the picture is revised somewhat. If, as has been suggested by Curry (1987), injecting animals does alter their arousal levels, then the adoption of a non-injected control group would have been a more appropriate methodology to employ. Consequently, although not obviously supporting the hypothesis that offspring (and presumable grandoffspring) behaviour patterns can be attributed to differing baseline arousal levels, the results of this study are not sufficient to dismiss the arousal hypothesis completely.

Indeed, when considering the arousal hypothesis in some more detail, there is some evidence that arousal differences between the offspring groups do exist and in particular that offspring of IC animals are more aroused than either their SC or SEC counterparts. These hypothesised differences in baseline arousal levels emerged from the second Skinner box study (chapter seven), in which increasing the stimulating properties of the reinforcer resulted in an adjustment of

[9] For example, maternal impoverishment appears to contribute most to differences in emotionality in the open field, enrichment having the greatest effect in the Hebb-Williams.

the relative bar press rates of the three offspring groups, consistent with predictions based on an inverted-U arousal-performance hypothesis. However, as is common with this thesis, few of the other test situations could be seen to fit neatly into an arousal framework. It is the present author's opinion, therefore, that arousal may be contributing, in part, to the offspring groups' behavioural differences, but that arousal cannot be seen as the *only* cause of these effects. Indeed, it should be reiterated that throughout this thesis there has been considerable hesitation in attributing a causal relationship between arousal and performance. This caution reflects the considerable ambiguity in the literature as to whether the inverted-U hypothesis (Yerkes and Dodson 1908) is causal or merely correlational (Neiss 1988; 1990; Anderson 1990). Moreover, Landers (1980) has stated that "the inverted-U hypothesis is not an explanation for the arousal-performance relationship, it merely posits that this relationship is curvilinear without explaining what internal state or process produces it" (p78). It may well be therefore, that the notion of differential arousal levels as a causal explanation for the offspring effects needs reassessing. In the remainder of this section the debates surrounding the construct of arousal will be highlighted and a way around these issues will be suggested. In particular, it will be argued that one avenue through the problems generated by both the arousal and stress hypotheses, would be to refine the level of analysis, exploring offspring and grandoffspring behaviour at a biochemical level.

Part of the problem facing the concept of arousal and more specifically the inverted-U hypothesis, according to Neiss (1988), is that it is effectively immune to falsification as "arousal cannot be created in a pure form for research purposes" (p354) in order to be tested. Indeed, even altering arousal levels by the use of chemicals such as amphetamine, as has been advocated by Walsh and Cummins (1975) and has been employed in this thesis and by other researchers (Einon and Sahakian 1979), is open to criticism (Neiss 1988; 1990). For example, as early as 1957, Miller urged caution in the interpretation of the results of drug studies because of pharmacological side effects. It may be that factors other than the physiological changes that are often subsumed under arousal, which result from the procedure of being injected with for example amphetamines, are themselves causing the behavioural changes observed. Futhermore, the con-

struct of arousal as defined via the inverted-U hypothesis invoked in this thesis, originally gained support because of its unidimensional nature (Duffy 1934; 1941; Malmo 1959). As psychophysiological and neurophysiological research has become more sophisticated, however, researchers have seen more utility in distinguishing between two or more forms of arousal (Neiss 1988), for example autonomic versus cortical arousal, or at a neurobiochemical level, arousal subserved by the sympathetic adrenal medullary system which is stimulated by the flight-fight emotions and the pituitary adrenal cortical sytem which is stimulated by anxiety (Henry 1976).

This redefinition of the construct of arousal, from one which is unidimensional and global to a more multifaceted and specific series of psychobiological states, has obvious implications for the inverted-U hypothesis. With arousal no longer seen as a unified construct, defining efficient performance in terms of optimal arousal levels no longer seems appropriate. However, this reconceptualisation of arousal (Neiss 1988; 1990) is not without its critics. Recently, for example, Anderson (1990) has argued that "although the ultimate value of the hypothetical, conceptual construct of arousal is as yet unresolved, substantial evidence does favour its pragmatic usefulness and hence its continued investigation" (p 99). This evidence can be found in the literature exploring the relationship between arousal and cognitive performance in which sophisticated methodologies allow the inverted-U hypothesis to be tested. Anderson (1990) has argued that under these conditions strong evidence for the inverted-U has been provided and suggests that the arousal-performance relationship may be altered by the nature of the functional domain being investigated.

Whatever the current state of conceptualisation of arousal, it seems clear that in the present thesis, the use of this construct as an explanation for both the behavioural differences observed in the offspring of differentially housed dams and as a possible mediating mechanism can no longer be considered as an appropriate levels of analysis. A more useful approach to employ in the investigation of both the nature of the offspring effects and their mediation in future studies, it is suggested, might be at the neurochemical and endocrinological levels. This suggestion is

posited for several reasons.

Firstly, the construct of arousal itself can be redefined and thus refined, in terms of the neurochemical systems which control it (Neiss 1988). For example, cortical arousal, it has been suggested, involves noradrenergic transmission (Tucker and Williamson 1984) and it may well be that the behavioural effects noted in the offspring are merely a reflection of altered neurochemical activity. Secondly, within the EC/IC literature itself are several reports of the effects of enrichment on an animal's neurochemistry (see chapter one, section 1:4) and in particular its noradrenergic systems. For example, norepinephrine (NE) has been implicated in certain phenomena that may be connected with EC/IC effects including arousal, learning and memory (Kety 1970), neural plasticity (Kasamatu et al 1981) and investigatory behaviour (Flicker and Geyer 1982). Furthermore, depletion of brain NE by injection of 6-hydroxydopamine reduces the EC/IC effects (Mirmiran et al 1983; O'Shea et al 1983; Pappas et al 1984; Pappas et al 1987) further implicating this neurotransmitter in the mediation of environmentally induced changes. Indeed, Pappas et al (1987) have suggested that NE "is permissive to the deleterious behavioural consequences of restricted experience during maturation" (p153). However, as Renner and Rosenzweig (1987) point out, the changes occuring in NE and behaviour say little about causality. Indeed, the role of NE in mediating the EC/IC effects is best seen as correlational rather than causal at this stage. Whatever the role of these neurotransmitters, of relevance to the present work is simply that if neurochemicals are having an effect in the parent generation, it is not unreasonable to suggest that they might also underly behavioural differences in the offspring and, taking this one stage further, possibly have a causal role to play in the observed offspring differences.

Indeed, within the prenatal stress literature, there is evidence that manipulating the mother has an impact on her offsprings' neurochemistry (see chapter three; section 3:3) and in particular the effects of prenatal stress have been found to alter the catecholamines norepinephrine (NE) and dopamine (DA) (Moyer, Herrenkohl and Jacobowitz 1978; Peters 1982; Fride et al 1985; Fride and Weinstock 1987). This is of interest given the postulated involvement of these neurotransmitters

in the regulation of cortical arousal. Furthermore, prenatal stress delays the development of noradrenergic neurons (Peters 1984) and the cerebral lateralisation of dopamine activity (Fride and Weinstock 1988). It may well be that, therefore, these neurotransmitters that are altered by both direct environmental experience and prenatal stress are also involved in the offspring effects.

One final reason for advocating a more micro level of analysis when trying to understand the nature of the offspring effects emerges from data presented in this thesis. In the animals exposed directly to SEC, SC and IC, behaviour varied according to the sex of the animal. Furthermore, sex differences also emerged in the offspring groups. It has been demonstrated elsewhere that in female animals at least, hormones interact with the effects of differential rearing (Diamond, Johnson and Ingham 1971; Hamilton, Diamond, Johnson and Ingham 1977; Hoover and Diamond 1976). In the prenatal literature reviewed in chapter three, manipulation of the mother produces different effects in male and female offspring. It may well be, therefore, that both the causes of the offspring effects and their mediation are linked to levels of circulating hormones rather than either baseline arousal levels [10] or stress. Indeed, in the few studies investigating the effects of enrichment on subsequent generations, the notion of hormonal mediation has been discussed when any attempt to explain the results has been made. For example, Kiyono et al (1985) talk rather vaguely about "maternal biochemical changes produced by enrichment" altering "the intrauterine environment of the fetuses" (p434) when trying to explain the differences in Hebb-Williams performance observed in their prenatally enriched and impoverished animals, whilst Diamond (1987) has speculated that progesterone may be involved [11]. In both of these studies, enrichment occured during pregnancy.

In the present work, of course, as the maternal manipulations occured prior to conception, the mechanisms may well be very different. However, of all the possible mechanisms suggested in

[10] This is not a new suggestion, Uphouse having advocated a re-apparaisal of the hormonal explanation of the EC/IC effects in 1980.

[11] The apparent mediation of the transfer of effects of differential environments across generations by progesterone, according to Diamond, reflects the fact that it can cross the blood-brain barrier.

this chapter and in chapters six and seven, in the author's opinion, it is the endocrinological and neurochemical levels of analysis which offer the most useful avenue for future investigation. Furthermore, more research on the functional significance of the sexually dimorphic behaviours noted in this thesis and in previous work may well be necessary before any conclusions about mediation can be drawn.

In summary, in this thesis clear differences between offspring and grandoffspring of differentially housed mothers were found. It has been suggested throughout this work that effects may be mediated in utero and/or in the mother-infant interactions in the period following birth to weaning. Possible mechanisms such as differential opportunities for learning, stress and/or arousal have been investigated directly (chapters seven and eight) and do not appear to be the appropriate levels of explanation as mediators of the effects. In this section, a more sophisticated micro level of analysis was advocated, with an emphasis being placed on neurochemical and endocrinological investigations being designed in the future. In the next section some more specific avenues of research are outlined, before conclusions from this present research are drawn.

9:5 AVENUES FOR FUTURE RESEARCH

The present work can be seen as contributing to the beginnings of a new area of research, which has progressed some way towards investigating the transfer of EC/IC effects across generations. There are, however, numerous questions which remain to be answered, which can be seen as falling within three general fields of inquiry.

Firstly, the nature of the offspring effects need to be considered further. In particular, in the present thesis, some indication of the interaction of environment with gender emerged. However, for both the grandoffspring groups and for the later studies investigating learning and arousal differences between the progeny of SEC, SC and IC dams, only male animals were employed. One avenue for future research, therefore, is quite simply to explore these sex differences further, as the functional significance of the sexual dimorphism noted in this thesis is as yet unresolved.

Secondly, this current work concentrated on a behavioural analysis of offspring and grandoffspring generations. Recently Kolb (1991) has advocated that new directions for research into recovery of function following brain damage should include both behavioural and neuroanatomical measures, to establish stronger correlational profiles. In the present author's opinion this trend in animal research should not be confined to investigations of recovery of function. Any investigations of the impact of experience should also take account of the wide ranging nature of this impact by simultaneously exploring brain and behaviour. In future research, therefore, both biochemical and behavioural measures should be made of offspring and grandoffspring generations. Moreover, given that both neurochemical and endocrine effects have been found in animals directly exposed to differential environments and that these changes may be involved in mediating the enrichment effects, it is recommended that these biochemical analyses form the focus of research in the near future.

Finally, one question which has not been addressed in this thesis, but which is of increasing theoretical importance in understanding the mediation of these effects, is the relative importance of prenatal as opposed to postpartum maternal influence on the offspring. In the present work, no attempt was made to separate out the contributions of these two periods of time to the intergenerational transfer of SEC, SC and IC effects [12]. Indeed, animals were deliberately left with their mothers postpartum to maximise any maternally induced effects. However, from a theoretical point of view, there is much to be learnt from experimentally distinguishing between prenatally and postnatally mediated effects. In particular, if the offspring effects are only seen in animals that have experienced some interaction with their mothers in the postpartum period, then a focus on that period alone may elucidate those conditions which are necessary for the transfer of effects to occur, which in turn may highlight the possible causes of said effects. Furthermore, throughout this thesis it has been suggested that maternal behaviours of differentially housed animals are qualitatively different from each other based on the findings of one study which has examined this type of behaviour (Muir et al 1985). It should be noted, however, that the

[12] The isolation of prenatal and postnatal effects is relatively simple to achieve, requiring that some litters be fostered, some cross-fostered and some left with their natural mothers.

maternal behaviours observed in this study were of a fairly gross nature, time spent in nest, time spent nursing, time spent nest building and time spent pup licking. A more detailed analysis of maternal behaviours should be undertaken in the future, both to extend the behavioural profile of enriched and impoverished animals and to pin-point possible differences in mother-infant interactions between SEC, SC and IC dams and their progeny to see whether it is these interactions which are important in the transfer of effects.

9:6 SOME FINAL THOUGHTS

In this thesis the impact of exposing an animal to a relatively "minimal" (McKim and Thompson 1975) environmental manipulation has been found to have an impact on the behaviour of both its offspring and grandoffspring. Typically in the literature, manipulation of the maternal generation has been quite "punitive" (McKim and Thompson 1975). In this thesis, however, it has become obvious that something as simple as changes in an animals' living conditions are enough to have an impact over several generations.

Furthermore, unlike previous research where the environmental experience has typically been given to a pregnant animal and can thus be seen as influencing the young organism from conception (Diamond et al 1971; 1984; Kiyono et al 1982; 1985; Inouye et al 1986), the present research extends the concept of early experience to include the experience of previous generations. After all, in this thesis, none of the offspring and grandoffspring groups was exposed to any form of direct environmental manipulation. The effects of SEC, SC and IC must then have been passed on from previous generations.

Given these findings, there are several implications that should be considered. Firstly, within the context of the nature/nurture debate, the present research can be seen as extending the impact of experience across generations. More specifically, the "nature" of an organism must now be seen as involving the interaction of its own experiences and genetic predispositions with a wider social environment, namely the experiences of its parent and grandparent generations. Secondly,

in Britain today, there is a growing interest in pre-conception counselling [13]. To date this has focused on nutritional and health care issues. One thought from the present work, however, is that some degree of awareness of the environmental conditions of prospective parents should also be incorporated into these counselling sessions.

Finally, the present thesis has some relevance to understanding the causes of enrichment effects in animals directly exposed to these types of environments. More specifically, if effects emerge in offspring which are similar to those reported in their parent generations, then it may be possible to eliminate those postulated causes of the EC/IC effects that could not explain the offspring effects. That is the offspring generation could be seen as a "filtering" device which can be used to highlight only those explanations which were of relevance to both generations.

[13] For example, Mothercare, one of the largest mother and baby care chains has produced a leaflet in conjunction with the Royal College of Midwives detailing a variety of preconception issues for parents. For further information the reader is refered to Sylvia Meredith Health Education Advisory Service, 3 Elgin Road, Sutton, Surrey SM1 3SN.

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APPENDICES TO EXPERIMENTAL CHAPTERS

APPENDIX: CHAPTER FIVE

EXPERIMENT ONE

OPEN FIELD

SOURCE	S.O.S	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Sex	60406.9	1	60406.9	20.75	0.00012
Environ	19813.2	2	9906.6	3.40	0.03937
Sex by Environ	9893.96	2	4946.98	1.70	0.19065
ERROR	157171.0	54	2910.58		
WITHIN SUBJECTS					
Days	10810.8	4	2702.70	4.84	0.00125
Days by Sex	1760.64	4	440.16	0.79	0.53591
Days by Environ	33522.7	8	4190.34	7.51	0.00000
Days by Environ by Sex	11838.8	8	1479.85	2.65	0.00873
ERROR	120578.0	216	558.23		
TOTAL	425796.0	299			

Summary table of ANOVA of number of lines crossed over five days of open field testing.

GROUP	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
SEC Male	105.40	97.50	88.30	68.70	87.80
SEC Female	151.70	139.00	98.30	103.50	109.20
IC Male	99.70	103.40	122.20	82.30	91.70
IC Female	111.20	140.50	157.80	147.50	147.70
SC Male	95.90	106.10	90.50	101.30	82.10
SC Female	112.50	115.70	111.00	110.10	92.90

Means of number of lines crossed over the five days of testing of the six experimental groups. (N=10 per group)

MEANS	SEC	104.00
	IC	120.47
	SC	101.81
MS ERROR	2910.58	
D.F.	54	
N PER GROUP	100	
CRITICAL DIFFERENCE	r=2	q(0.05)=15.26
		q(0.01)=20.28
	r=3	q(0.05)=18.34
		q(0.01)=23.09
COMPARISON	SEC vs IC	p<0.05
	SC vs IC	p<0.05
	SEC vs SC	N/S

Summary of Newman Keuls carried out on lines crossed data for the three groups over all days of testing.

MEANS	SEC IC SC	128.55 105.45 104.20
MS ERROR	558.23	
D.F.	216	
N PER GROUP	20	
CRITICAL DIFFERENCE	r=2 r=3	q(0.05)=14.63 q(0.01)=19.23 q(0.05)=17.48 q(0.01)=21.76
COMPARISON	SEC vs IC SEC vs SC SC vs IC	p<0.01 p<0.01 N/S

Summary table of Newman Keuls performed on the day one lines crossed data for the three groups, collapsed by sex. (N=20 per group)

MEANS	SEC FEMALES SEC MALES SC FEMALES SC MALES IC FEMALES IC MALES	120.34 89.54 108.44 95.18 140.94 99.86
MS ERROR	2910.58	
D.F.	54	
N PER GROUP	50	
CRITICAL DIFFERENCE	r=2 r=3 r=4 r=5 r=6	q(0.05)=20.77 q(0.01)=27.60 q(0.05)=24.96 q(0.01)=31.42 q(0.05)=27.45 q(0.01)=33.69 q(0.05)=29.21 q(0.01)=35.38 q(0.05)=30.54 q(0.01)=36.63

Summary tables of Newman Keuls performed on the number of lines crossed by the six experimental groups over the five days of open field testing, comparisons to follow.

GROUP	SEC Female	SEC Male	SC Female	SC Male	IC Female	IC Male
SEC Female						
SEC Male	0.05					
SC Female	N/S	N/S				
SC Male	N/S	N/S	N/S			
IC Female	N/S	0.01	0.01	0.01		
IC Male	N/S	N/S	N/S	N/S	0.01	

Summary table of Newman Keuls comparisons made between the six experimental groups over the five days of open field testing.

SOURCE	S.O.S	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Sex	836.67	1	836.67	7.63	0.00776
Environ	693.85	2	346.92	3.16	0.04877
Sex by Environ	199.87	2	99.93	0.91	0.41053
ERROR	5919.26	54	109.62		
WITHIN SUBJECTS					
Days	987.25	4	246.81	12.48	0.00000
Days by Sex	185.02	4	46.25	2.34	0.05535
Days by Environ	1032.49	8	129.06	6.53	0.00001
Days by Environ by Sex	104.00	8	13.00	0.66	0.73013
ERROR	4270.44	216	19.77		
TOTAL	14228.8	299			

Summary table of ANOVA of number of rears over five days of open field testing.

GROUP	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
SEC Male	10.50	11.10	9.90	9.60	14.90
SEC Female	18.00	17.50	14.40	14.40	19.10
IC Male	12.50	11.70	10.80	7.30	9.70
IC Female	16.70	12.70	12.70	12.70	12.30
SC Male	14.30	9.70	6.70	8.70	7.90
SC Female	20.50	9.70	7.30	11.30	6.10

Means of number of rears over the five days of testing of the six experimental groups. (N=10 per group)

MEANS	SEC	13.94
	IC	11.91
	SC	10.22
MS ERROR	109.62	
D.F.	54	
N PER GROUP	100	
CRITICAL DIFFERENCE	r=2	q(0.05)=2.96
		q(0.01)=3.55
	r=3	q(0.05)=3.93
		q(0.01)=4.48
COMPARISON	SEC vs IC	N/S
	SEC vs SC	N/S
	IC vs SC	N/S

Summary table of Newman Keuls performed on the rearing data.

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Sex	4.81	1	4.81	0.80	0.37935
Environ	17.69	2	8.84	1.47	0.23869
Sex by Environ	6.89	2	3.44	0.57	0.57367
ERROR	325.80	54	6.03		
WITHIN SUBJECTS					
Days	10.95	4	2.74	0.68	0.61003
Days by Sex	20.55	4	5.14	1.27	0.28009
Days by Environ	53.95	8	6.74	1.67	0.10590
Days by Environ by Sex	24.15	8	3.02	0.75	0.65003
ERROR	870.80	216	4.03		
TOTAL	1335.59	299			

Summary table of ANOVA of seconds spent in the centre circle of the open field over five days of testing.

GROUP	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
SEC Males	2.10	0.70	0.80	2.70	2.20
SEC Females	1.90	2.20	1.40	1.30	1.20
IC Males	1.50	1.10	2.50	1.20	1.50
IC Females	1.10	2.20	3.20	1.60	2.90
SC Males	1.70	0.60	1.10	1.50	1.00
SC Females	2.50	0.90	1.50	0.70	1.40

Means of seconds spent in the centre of the open field over the five days of testing for the six experimental groups. (N=10 per group.)

SOURCE	S.O.S	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Sex	46.4133	1	46.4133	11.32	0.00177
Environ	14.1267	2	7.06333	1.72	0.8638
Sex by Environ	14.1267	2	7.06334	1.72	0.18638
ERROR	221.320	54	4.09852		
WITHIN SUBJECTS					
Days	8.02000	4	2.00500	2.26	0.06311
Days by Sex	8.02000	4	2.00500	2.26	0.06311
Days by Environ	17.8400	8	2.23000	2.51	0.01259
Days by Environ by Sex	17.8400	8	2.23000	2.51	0.01259
ERROR	191.882	216	0.88834		
TOTAL	539.590	299			

Summary table of ANOVA of number of defecations over the five days of testing in the open field.

GROUP	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
SEC Male	1.60	2.80	0.50	1.60	0.50
SEC Females	0.00	0.00	0.00	0.00	0.00
IC Males	0.00	0.60	0.60	1.30	0.00
IC Females	0.00	0.00	0.00	0.00	0.00
SC Males	0.10	0.30	0.50	0.50	0.90
SC Females	0.00	0.00	0.00	0.00	0.00

Means of numbers of defeactions over the five days of testing, for the six experimental groups. (N=10 per group.)

SKINNER BOX

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Sex	692653	1	692653	15.46	0.00047
Environ	190920	2	95460.1	2.13	0.12675
Sex by Environ	40223.2	2	20111.6	0.45	0.64643
ERROR	2.42004E6	54	44815.6		
WITHIN SUBJECTS					
Days	4.27944E6	5	855888.0	94.99	0.00000
Days by Sex	434611	5	86922.2	9.65	0.00000
Days by Environ	152986	10	15298.6	1.70	0.08066
Days by Environ by Sex	206993	10	20699.3	2.30	0.01333
ERROR	2.43268E6	270	9009.92		
TOTAL	1.08505E7	359			

Summary table of ANOVA of numbers of bar presses over the six days of Skinner box testing.

GROUP	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6
SEC Males	23.70	34.40	130.80	211.60	288.40	332.30
SEC Females	32.70	31.20	90.90	121.00	127.30	189.40
IC Males	36.00	83.50	185.90	265.70	342.00	454.40
IC Females	34.40	43.00	79.20	132.00	274.20	359.80
SC Males	20.60	64.60	149.50	267.80	313.50	524.10
SC Females	44.60	42.60	80.60	115.50	186.40	164.90

Means of numbers of bar presses over the six days of Skinner box testing for the six experimental groups. (N=10 per group)

MEANS	SEC MALE	332.30
	SEC FEMALE	189.40
	IC MALE	454.40
	IC FEMALE	359.80
	SC MALE	524.10
	SC FEMALE	164.90
MS ERROR	9009.92	
D.F.	270	
N PER GROUP	10	
CRITICAL DIFFERENCE	r=2	q(0.05)=83.14
		q(0.01)=109.26
	r=3	q(0.05)=99.35
		q(0.05)=123.66
	r=4	q(0.05)=108.96
		q(0.01)=132.07
	r=5	q(0.05)=115.86
		q(0.01)=138.07
	r=6	q(0.05)=120.96
		q(0.01)=142.87

GROUP	SEC Male	SEC Female	IC Male	IC Female	SC Male	SC Female
SEC Male						
SEC Female	0.01					
IC Male	0.05	0.01				
IC Female	N/S	0.01	N/S			
SC Male	0.01	0.01	N/S	0.01		
SC Female	0.01	N/S	0.01	0.01	0.01	

Summary tables of a) Newman Keuls performed on the number of bar presses of the six experimental groups on the last day of Skinner box testing and b) comparisons made between the groups.

VISUAL CLIFF

CHI SQUARE DISTRIBUTION

DEPTH	SEC	SC	IC
SHALLOW	O=12	O=12	O=11
DEEP	O=8	O=8	O=9
χ^2=0.13714	d.f.=2	p>0.05	N/S

Table of observed (O) frequencies for choice made by the three groups (males and females combined N=20 per group) in the visual cliff when the deep side was set at one inch. The χ^2 distribution was not significant, that is there was no evidence of any association between environmental experiencce and shallow/deep choice preference when the cliff was set at 1 inch.

DEPTH	SEC	SC	IC
DEEP	O=13	O=16	O=18
SHALLOW	O=7	O=4	O=2
χ^2=3.73159	d.f.=2	p>0.05	N/S

Table of observed frequencies for choice made by the three groups (N=20 per group) in the visual cliff when the deep side was set at 12 inches. The χ^2 distribution was not significant.

LATENCY TO DESCEND DATA

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Sex	42.01	1	42.01	0.22	0.64513
Environ	1991.22	2	995.61	5.23	0.00848
Sex by Environ	303.82	2	151.91	0.80	0.45903
ERROR	10273.1	54	190.24		
WITHIN SUBJECTS					
Trials	33.08	1	33.08	0.29	0.59834
Trials by Sex	15.41	!	15.41	0.14	0.71364
Trials by Environ	162.45	2	81.23	0.71	0.49842
Trials by Environ by Sex	298.32	2	149.16	1.31	0.27711
ERROR	6140.24	54	113.71		
TOTAL	19259.6	119			

Summary table of ANOVA of latency to descend onto the visual cliff, for both trials.

GROUP	TRIAL 1 (1 INCH)	TRIAL 2 (12 INCH)
SEC Males	4.60	6.50
SEC Females	4.00	5.70
IC Males	8.60	12.40
IC Females	15.90	10.00
SC Males	21.00	14.30
SC Females	12.90	11.80

Means of the latencies to descend onto the visual cliff, for the two trials of the six experimental groups. (N=10 per group)

MEANS	SEC	5.20
	IC	11.72
	SC	15.00
MS ERROR	190.24	
D.F.	54	
N PER GROUP	40	
CRITICAL DIFFERENCE	r=2	q(0.05)=6.17
		q(0.01)=8.19
	r=3	q(0.05)=7.41
		q(0.01)=9.33
COMPARISON	SEC vs IC	p<0.05
	SEC vs SC	p<0.01
	SC vs IC	N/S

Summary table of Newman Keuls performed on the latency to descend data.

EXPERIMENT TWO

OPEN FIELD

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Environ	65776.1	2	32888.1	15.53	0.00014
ERROR	50820.0	24	2117.50		
WITHIN SUBJECTS					
Days	3050.18	4	762.54	2.37	0.05676
Days by Environ	6517.43	8	814.68	2.54	0.01503
ERROR	30849.9	96	321.35		
TOTAL	157014.0	134			

Summary table of ANOVA of number of lines crossed over five days of open field testing.

GROUP	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
SEC Females	99.00	82.33	91.56	90.22	81.67
IC Females	119.78	144.11	145.56	151.11	145.89
SC Females	121.11	116.56	130.11	136.22	130.44

Means of numbers of lines crossed over the five days of open field testing, for the three post partum groups. (N=9 per group)

MEANS	SEC	88.96
	IC	141.29
	SC	126.89
MS ERROR	2117.5	
D.F.	24	
N PER GROUP	45	
CRITICAL DIFFERENCE	r=2	q(0.05)=20.03
		q(0.01)=27.16
	r=3	q(0.05)=24.21
		q(0.01)=31.21
COMPARISON	SEC vs SC	p<0.01
	SEC vs IC	p<0.01
	SC vs IC	N/S

Summary table of Newman Keuls performed on lines crossed data.

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
ENVIRON	3246.33	2	1623.16	12.75	0.00033
ERROR	3056.27	24	127.34		
WITHIN SUBJECTS					
Days	291.66	4	72.91	1.80	0.13467
Days by Environ	305.45	8	38.18	0.94	0.48760
ERROR	3895.29	96	40.58		
TOTAL	10795.0	134			

Summary table of ANOVA of number of rears over five days of open field testing.

GROUP	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
SEC Females	17.89	12.89	12.89	13.44	15.11
IC Females	27.44	27.00	25.33	28.00	23.44
SC Females	25.44	25.11	21.33	18.56	21.00

Means of numbers of rears for the three post partum groups over the five days of open field testing. (N=9 per group.)

MEANS	SEC	14.44
	IC	26.24
	SC	22.29
MS ERROR	127.34	
D.F.	24	
N PER GROUP	45	
CRITICAL DIFFERENCE	r=2	q(0.05)=4.91
		q(0.01)=6.66
	r=3	q(0.05)=5.94
		q(0.05)=7.65
COMPARISON	SEC vs IC	$p<0.01$
	SEC vs SC	$p<0.01$
	SC vs IC	N/S

Summary table of Newman Keuls performed on the rearing data.

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Environ	224.04	2	112.02	3.29	0.05324
ERROR	816.49	24	34.02		
WITHIN SUBJECTS					
Days	111.75	4	27.94	2.12	0.08323
Days by Environ	195.59	8	24.45	1.86	0.07568
ERROR	1265.07	96	13.18		
TOTAL	2612.93	134			

Summary table of ANOVA of time spent in the centre circle of the open field over five days of testing.

GROUP	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
SEC Females	7.33	4.11	5.44	4.33	2.44
IC Females	7.44	7.33	6.67	8.56	9.44
SC Females	9.33	7.33	5.22	5.33	4.33

Means of time spent in the centre circle of the open field for the three post partum groups. (N=9 per group)

MEANS	SEC IC SC	4.73 7.89 6.31
MS ERROR	34.02	
D.F.	24	
N PER GROUP	45	
CRITICAL DIFFERENCE	r=2 r=3	q(0.05)=2.53 q(0.01)=3.44 q(0.05)=3.06 q(0.01)=3.95
COMPARISON	SEC vs IC SEC vs SC SC vs IC	$p<0.05$ N/S N/S

Summary table of Newman Keuls performed on the time in centre data.

APPENDIX: CHAPTER SIX

EXPERIMENT ONE

LITTERSIZE

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Sex	0.01	1	0.01	0.00	0.92415
Environ	10.95	2	5.48	0.70	0.50253
Sex by Environ	0.02	2	0.01	0.00	0.99083
ERROR	889.95	114	7.81		
TOTAL	900.92	119			

Summary table of ANOVA of the littersize of each subject employed in experiment one, by sex and experimental group.

GROUP	MEAN
SEC Male	10.25
SEC Female	10.30
SC Male	10.20
SC Female	10.20
IC Male	9.60
IC Female	9.60

Means of the six offspring groups' littersizes. (N=20 per group)

WEANING WEIGHTS

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
Sex	64.53	1	64.53	0.69	0.41286
Environ	177.22	2	88.61	0.95	0.39275
Sex by Environ	11.72	2	5.86	0.06	0.93000
ERROR	10658.0	114	93.49		
TOTAL	10911.5	119			

Summary table of ANOVA of weaning weights of the six offspring groups, by sex and experimental background.

GROUP	MEAN
SEC Male	42.45
SEC Female	41.85
SC Male	43.55
SC Female	41.80
IC Male	40.90
IC Female	38.85

Means of the six offspring groups' weaning weights. (N=20 per group)

OPEN FIELD

LINES CROSSED

SOURCE	S.O.S	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Sex	354.20	1	354.20	0.15	0.70036
Environ	5836.52	2	2918.26	1.23	0.29445
Sex by Environ	8239.00	2	4119.50	1.74	0.17775
ERROR	269513.00	114	2364.15		
WITHIN SUBJECTS					
Days	8673.91	4	2168.48	3.26	0.01196
Days by Sex	3965.60	4	991.40	1.49	0.20309
Days by Environ	19334.1	8	2416.77	3.63	0.00064
Days by Environ by Sex	1302.58	8	162.82	0.24	0.98119
ERROR	303557.00	456	665.69		
TOTAL	620776.00	599			

Summary table of ANOVA of number of lines crossed over five days of open field testing.

GROUP	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
SEC Male	65.35	64.40	82.55	63.45	80.15
SEC Female	63.35	85.00	80.90	71.00	93.60
IC Male	78.65	80.50	78.60	72.40	83.80
IC Female	77.05	91.80	81.15	81.25	92.30
SC Male	91.85	92.80	88.70	82.15	73.85
SC Female	78.15	86.95	80.20	73.50	66.05

Means of number of lines crossed over the five days of testing of the six experimental groups. (N=20 per group)

MEANS	SEC SC IC	64.35 85.00 77.85
MS ERROR	665.69	
D.F.	456	
N PER GROUP	40	
CRITICAL DIFFERENCE	r=2 r=3	q(0.05)=11.30 q(0.01)=14.84 q(0.05)=13.50 q(0.05)=16.80
COMPARISON	SEC vs IC SEC vs SC SC vs IC	p<0.05 p<0.01 N/S

Summary of Newman Keuls performed on the number of lines crossed on day one of open field testing for the three offspring groups.

REARS

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Sex	256.11	1	256.11	4.17	0.04097
Environ	1020.81	2	510.41	8.30	0.00071
Sex by Environ	95.04	2	47.52	0.77	0.46812
ERROR	7008.84	114	61.48		
WITHIN SUBJECTS					
Days	746.13	4	186.53	9.92	0.00001
Days by Sex	136.13	4	34.03	1.81	0.12446
Days by Environ	575.96	8	71.99	3.83	0.00040
Days by Sex by Environ	79.93	8	9.99	0.53	0.83374
ERROR	8571.06	456	18.80		
TOTAL	18490.0	599			

Summary table of ANOVA of number of lines crossed by the six offspring groups (three environmental backgrounds, two sexes) over the five days of open field testing.

GROUP	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
SEC Male	4.55	3.90	4.35	6.30	8.10
SEC Female	4.05	5.60	6.25	6.40	10.05
SC Male	7.15	6.40	5.45	5.05	7.35
SC Female	6.75	6.80	6.70	6.35	6.35
IC Male	7.45	5.50	8.20	6.80	10.70
IC Female	6.75	8.90	11.65	9.80	13.50

Mean number of rears for the six offspring groups over the five days of open field testing.

MEANS	SEC SC IC	5.95 6.42 8.92
MS ERROR	61.48	
D.F.	114	
N PER GROUP	200	
CRITICAL DIFFERENCE	r=2 r=3	q(0.05)=1.55 q(0.01)=2.05 q(0.05)=1.86 q(0.01)=2.32
COMPARISON	SEC vs IC SEC vs SC SC vs IC	p<0.01 N/S p<0.01

Summary table of Newman Keuls performed on the number of rears data.

TIME IN CENTRE

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Sex	0.67	1	0.67	0.06	0.79714
Environ	84.76	2	42.38	3.66	0.02793
Sex by Environ	23.09	2	11.55	1.00	0.37355
ERROR	1319.58	114	11.58		
WITHIN SUBJECTS					
Days	181.02	4	45.25	6.89	0.00009
Days by Sex	22.32	4	5.58	0.85	0.49617
Days by Environ	135.02	8	16.88	2.57	0.00958
Days by Sex by Environ	50.42	8	6.30	0.96	0.46748
ERROR	2993.63	456	6.56		
TOTAL	4810.51	599			

Summary table of ANOVA of time spent in the centre of the open field by the six offspring groups over the five days of testing.

GROUP	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
SEC Male	0.30	2.20	2.65	2.40	4.50
SEC Female	1.20	1.95	3.25	2.45	3.30
SC Male	1.70	2.95	3.60	3.10	1.95
SC Female	0.85	2.00	2.35	2.70	2.50
IC Male	0.60	2.15	1.75	0.75	1.85
IC Female	1.95	2.05	1.25	2.05	1.80

Mean number of seconds spent in the centre of the open field by the six offspring groups over the five days of testing. (N=20 per group)

MEANS	SEC SC IC	2.45 2.38 1.62
MS ERROR	11.58	
D.F.	114	
N PER GROUP	200	
CRITICAL DIFFERENCE	r=2 r=3	q(0.05)=0.67 q(0.01)=0.89 q(0.05)=0.80 q(0.01)=1.01
COMPARISON	SEC vs IC SEC vs SC SC vs IC	p<0.05 N/S p<0.05

Summary table of Newman Keuls performed on the time in centre data.

DEFECATIONS

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Sex	19.8016	1	19.8016	3.66	0.05498
Environ	2.52334	2	1.26167	0.23	0.79399
Sex by Environ	4.40337	2	2.20168	0.41	0.67216
ERROR	616.390	114	5.40693		
WITHIN SUBJECTS					
Days	14.7767	4	3.69417	2.26	0.06131
Days by Sex	3.65670	4	0.91417	0.56	0.69653
Days by Environ	14.5933	8	1.82417	1.11	0.35190
Days by Sex by Environ	7.01328	8	0.87666	0.54	0.83087
ERROR	746.767	456	1.63765		
TOTAL	1429.93	599			

Summary table of ANOVA of the number of defecations in the open field by the six offspring groups over the five days of testing.

GROUP	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
SEC Male	0.50	0.95	0.70	0.90	0.95
SEC Female	0.20	0.45	1.10	0.35	0.65
SC Male	0.70	1.30	0.85	0.65	0.45
SC Female	0.65	0.80	0.60	0.50	0.40
IC Male	0.70	1.10	1.25	0.95	1.25
IC Female	0.35	0.35	0.80	0.65	0.35

Mean number of defecations in the open field by the six offspring groups over the five days of testing.

VISUAL CLIFF

CHI SQUARE DISTRIBUTION

DEPTH	SEC	SC	IC
SHALLOW	O=13	O=12	O=9
	E=10	E=10	E=10
DEEP	0=7	O=8	0=11
	E=10	E=10	E=10
χ^2=1.7652	d.f.=2	p>0.05	N/S

Table of observed (O) and expected (E) frequencies for choice made by the three offspring groups (N=20 per group) in the visual cliff when the "deep" side was set at 1 inch. The χ^2 distribution was not significant, that is there was no evidence of any association between the environmental background of the offspring and their shallow/deep choice preference when the cliff was set at 1 inch.

DEPTH	SEC	SC	IC
SHALLOW	O=13	O=11	0=6
	E=10	E=10	E=10
DEEP	O=7	O=9	O=14
	E=10	E=10	E=10
χ^2=5.2	D.F.=2	p>0.05	N/S

Table of observed (O) and expected (E) frequencies for choice made by the three offspring groups (N=20 per group) in the visual cliff when the "deep" side was set at 12 inches. The χ^2 distribution was not significant, that is there was no evidence of any association between the environmental background of the offspring and their shallow/deep choice preference when the cliff was set at 12 inches.

LATENCY TO DESCEND ONTO CLIFF

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Sex	340.03	1	340.03	1.78	0.188
Sex by Environ	611.67	2	305.83	1.60	0.211
Environ	328.07	2	164.03	0.86	0.429
ERROR	10313.70	54	190.99		
WITHIN SUBJECTS					
Cliff Depth	0.13	1	0.13	0.00	0.971
Environ by Cliff Depth	906.47	2	453.23	4.41	0.017
Sex by Cliff Depth	50.70	1	50.70	0.49	0.485
Sex by Environ by Cliff	428.60	2	214.30	2.09	0.134
ERROR	5546.10	54	102.71		
TOTAL	18625.47	119			

Summary of ANOVA table of time taken by the six offspring groups (males and females by three environmental backgrounds) to descend onto the visual cliff for the two cliff depths (1 and 12 inches).

GROUP	CLIFF DEPTH	MEAN
SEC	1	5.30
SEC	12	11.20
SC	1	15.65
SC	12	8.60
IC	1	8.90
IC	12	10.50

Mean latency to descend scores (in seconds) of the three offspring groups (males and females combined) when the visual cliff was set at both 1 and 12 inches.

SKINNER BOX

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Sex	114775.0	1	114775.0	5.33	0.02340
Environ	152188.0	2	76094.2	3.53	0.03511
Sex by Environ	7140.91	2	3570.45	0.17	0.84587
ERROR	1.16321E6	54	21541.0		
WITHIN SUBJECTS					
Days	785207.0	5	157041.0	37.71	0.00000
Days by Sex	117532.0	5	23506.3	5.64	0.00016
Days By Environ	123013.0	10	12301.3	2.95	0.00183
Days by Sex by Environ	16984.4	10	1698.44	0.41	0.94221
ERROR	1.12434E6	270	4164.20		
TOTAL	3.60439E6	359			

Summary of ANOVA of nember of bar presses for the six offspring groups, over the six days of training.

GROUP	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6
SEC Male	28.4	25.3	31.6	64.9	83.3	138.5
SEC Female	21.9	27.7	17.0	30.0	44.1	57.1
SC Male	35.4	30.1	60.4	87.0	203.1	249.0
SC Female	27.7	24.0	41.5	83.1	149.5	160.4
IC Male	25.2	18.5	29.7	71.7	158.8	199.8
IC Female	17.5	17.5	24.3	35.0	54.4	65.2

Mean number of bar presses of the six offspring groups over the six days of Skinner box training.

MEANS	SEC	47.48
	SC	95.93
	IC	59.80
MS ERROR	21541.0	
D.F.	54	
N PER GROUP	120	
CRITICAL DIFFERENCE	r=2	q(0.05)=37.91
		q(0.01)=50.37
	r=3	q(0.05)=45.55
		q(0.01)=57.34
COMPARISON	SEC vs IC	N/S
	SEC vs SC	p<0.05
	SC vs IC	N/S

Summary table of Newman Keuls performed on the Skinner box data.

EXPERIMENT TWO

LITTERSIZE

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Environ	48.07	2	24.03	2.03	0.14939
ERROR	319.80	27	11.84		
TOTAL	367.87	29			

Summary table of ANOVA of littersizes of the grandoffspring groups. (N=10 per group)

GROUP	MEAN
SEC	8.50
SC	11.40
IC	10.90

Mean littersize of the three grandoffspring groups. (N=10 per group)

WEANING WEIGHTS

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Environ	87.80	2	43.90	0.853	0.437
ERROR	1389.40	27	51.459		
TOTAL	1477.20	29	50.938		

Summary table of ANOVA of grandoffspring weaning weights. (N=10 per group)

GROUP	MEAN
SEC	52.20
SC	48.10
IC	50.90

Mean weaning weight of the three grandoffspring groups. (N=10 per group)

OPEN FIELD

LINES CROSSED

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Environ	14203.7	2	7101.86	2.67	0.08564
ERROR	71722.7	27	2656.40		
WITHIN SUBJECTS					
Days	10412.0	4	2603.01	6.07	0.00037
Environ by Days	3952.42	8	494.05	1.15	0.33411
ERROR	46282.0	108	428.54		
TOTAL	146573.0	149			

Summary table of ANOVA of number of lines crossed by the three grandoffspring groups over the five days of open field testing. (N=10 per group)

GROUP	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
SEC	80.00	90.60	84.40	87.40	64.90
SC	71.30	100.00	83.10	72.30	61.30
IC	66.20	63.20	56.90	60.10	49.40

Mean number of lines crossed in the open field of the three grandoffspring groups. (N=10 per group)

REARS

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Environ	335.05	2	167.53	5.44	0.01030
ERROR	830.82	27	30.77		
WITHIN SUBJECTS					
Days	204.37	4	51.09	5.72	0.00055
Days by Environ	211.15	8	26.39	2.95	0.00527
ERROR	965.28	108	8.94		
TOTAL	2546.67	149			

Summary table of ANOVA of number of rears for the three grandoffspring groups over the five days of open field testing.

GROUP	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
SEC	7.70	4.90	7.80	11.40	5.20
SC	2.50	4.10	5.00	6.00	3.40
IC	3.60	3.70	2.80	5.20	6.00

Mean number of rears over the five days of open field testing for the three grandoffspring groups. (N=10 per group)

MEANS	SEC SC IC	7.40 4.20 4.26
MS ERROR	30.77	
D.F.	27	
N PER GROUP	50	
CRITICAL DIFFERENCE	r=2 r=3	q(0.05)=2.26 q(0.01)=3.05 q(0.05)=2.73 q(0.01)=3.49
COMPARISON	SEC vs IC SEC vs SC SC vs IC	p<0.01 p<0.05 N/S

Summary table of the Newman Keuls performed on the number of rears in the open field of the three grandoffspring groups.

TIME IN CENTRE

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Environ	0.86333	2	0.43167	0.03	0.74976
ERROR	39.41000	27	1.45963		
WITHIN SUBJECTS					
Days	13.5233	4	3.38083	2.39	0.05435
Environ by Days	8.78666	8	1.09833	0.78	0.62528
ERROR	152.690	108	1.41380		
TOTAL	215.274	149			

Summary table of ANOVA of time spent in the centre circle of the open field over the five days of testing by the three grandoffspring groups. (N=10 per group)

GROUP	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
SEC	0.10	0.95	1.15	0.70	0.55
SC	0.80	1.00	1.25	0.15	0.35
IC	0.10	0.40	1.05	0.95	0.20

Mean number of seconds spent in the centre of the open field by the three grandoffspring groups, over the five days of open field testing. (N=10 per group)

DEFECATIONS

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Environ	1.56000	2	0.78000	0.58	0.57393
ERROR	36.5800[illegible]	27	1.35485		
WITHIN SUBJECTS					
Days	4.44000	4	1.11000	1.73	0.14766
Days by Environ	5.84000	8	0.73000	1.14	0.34406
ERROR	69.32000	108	0.64185		
TOTAL	117.740	149			

Summary table of ANOVA of number of defecations in the open field by the three grandoffspring groups over the five days of testing. (N=10 per group)

GROUP	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
SEC	0.40	0.00	0.00	0.30	0.90
SC	0.80	0.00	0.30	0.00	0.20
IC	0.20	0.00	0.00	0.20	0.00

Mean number of defecations for the three grandoffspring groups over the five days of open field testing. (N=10 per group)

SKINNER BOX

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Environ	1.05524E6	2	527618.0	1.93	0.16332
ERROR	7.38808E6	27	273633.0		
WITHIN SUBJECTS					
Days	1.52935E7	13	1.1762E6	59.49	0.00000
Days by Environ	619734.0	26	23835.9	1.21	0.22688
ERROR	6.94115E6	351	19775.4		
TOTAL	3.12977E7	419			

Summary table of ANOVA of number of bar presses for the three grandoffspring groups over the fourteen days of Skinner box training. (N=10 per group)

DAY	SCHEDULE	SEC	SC	IC
1	CRF	29.3	23.7	14.1
2	CRF	47.5	23.7	16.2
3	FR3	32.6	30.7	42.5
4	FR3	55.3	765.4	48.1
5	FR3	140.7	104.7	69.4
6	AD LIB			
7	AD LIB			
8	FR3	198.3	142.7	71.4
9	FR6	258.4	209.7	165.4
10	FR6	321.6	277.4	196.3
11	FR6	420.9	385.6	257.6
12	FR6	478.7	470.5	302.6
13	AD LIB			
14	AD LIB			
15	FR12	528.3	521.1	353.7
16	FR12	515.6	497.7	338.0
17	FR12	610.3	568.8	390.9
18	FR12	631.3	635.0	377.8

Mean number of bar presses over the eighteen days of Skinner box training for the three grandoffspring groups. (N=10 per group)

APPENDIX: CHAPTER SEVEN

EXPERIMENT ONE

HEBB-WILLIAMS: LITTERSIZE

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Environ	4.05	2	2.03	0.18	0.83715
ERROR	411.69	36	11.44		
TOTAL	415.74	38			

Summary table of ANOVA of the littersize of each subject employed in the Hebb-Williams maze for the three offspring groups.

GROUP	MEAN
SEC	9.08
SC	9.62
IC	9.85

Means of the three offspring groups' littersizes of the animals used in the Hebb-Williams maze. (N=13 per group)

HEBB-WILLIAMS: PRE-TRAINING

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Environ	92354.09	2	46177.04	3.66	0.036
ERROR	454753.15	36	12632.03		
WITHIN SUBJECTS					
Trials	673951.61	3	224650.54	59.43	0.000
Trials by Environ	44731.91	6	7455.32	1.97	0.076
ERROR	408248.23	108	3780.08		
TOTAL	1674039.0	155			

Summary table of ANOVA of time to eat pellets in the goal box over four trials in the first two days of pre-training.

GROUP	TRIAL 1	TRIAL 2	TRIAL 3	TRIAL 4
SEC	288.846	148.385	100.769	65.538
SC	283.077	239.308	176.231	140.154
IC	258.154	196.462	121.462	111.538

Means of the three offspring groups' time to eat pellets in the goal box in the first two days of pretraining, that is over four trials.

MEANS	SEC IC SC	150.8045 171.904 209.6925
MS ERROR	12632.03	
D.F.	36	
N PER GROUP	52	
CRITICAL DIFFERENCE	r=2 r=3	q(0.05)=45.04 q(0.01)=60.62 q(0.05)=54.39 q(0.01)=72.31
COMPARISON	SEC vs IC SEC vs SC SC vs IC	N/S p<0.05 N/S

Summary table of Newman Keuls performed on the latency to eat pellets data.

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Environ	60882.83	2	30441.41	2.49	0.097
ERROR	439297.10	36	12202.70		
WITHIN SUBJECTS					
Trials	52184.79	5	10436.96	3.68	0.003
Trials by Environ	34435.17	10	3443.52	1.21	0.285
ERROR	510549.21	180	2836.38		
TOTAL	5692749.1	233			

Summary table of ANOVA of time to leave the start box on days 3,4 and 5 of pre-training for the three offspring groups, each animal receiving two trials a day.

GROUP	TRIAL 1	TRIAL 2	TRIAL 3	TRIAL 4	TRIAL 5	TRIAL 6
SEC	4.15	24.00	3.84	20.53	3.15	9.92
SC	9.46	30.76	24.38	22.15	13.30	75.15
IC	41.00	61.61	57.92	16.46	33.69	91.76

Mean latency to leave the start box for the three offspring groups over the six pre-training runway trials.

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Environ	135456.10	2	67728.05	2.33	0.112
ERROR	1046137.23	36	29059.37		
WITHIN SUBJECTS					
Trials	537161.38	5	107432.28	19.56	0.000
Trials by Environ	57096.36	10	5709.64	1.04	0.412
ERROR	988462.92	180	5491.46		
TOTAL	2764314.0	233			

Summary table of ANOVA of time to reach the goal box on days 3, 4 and 5 of pre-training for the three offspring groups, each animals having two trials per day.

GROUP	TRIAL 1	TRIAL 2	TRIAL 3	TRIAL 4	TRIAL 5	TRIAL 6
SEC	142.23	105.54	43.30	27.76	5.61	12.00
SC	154.76	144.23	85.46	36.69	28.30	105.61
IC	185.08	155.93	98.76	51.15	48.46	147.08

Mean latency to reach the goal box for the three offspring groups over the six pre-training runway trials (days 3, 4 and 5: two trials a day).

HEBB-WILLIAMS: TRAINING

SOURCE	S.O.S	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Environ	43.744	2	21.872	2.775	0.076
ERROR	283.692	36	7.880		
TOTAL	327.436	38	8.617		

Summary table of ANOVA of number of days to reach criterion in the training mazes.

GROUP	MEAN
SEC OFFSPRING	4.77
SC OFFSPRING	6.85
IC OFFSPRING	7.15

Mean number of days taken by the three offspring groups to reach criterion in the training mazes. (N=13 per group)

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Environ	1370.98	2	685.49	2.35	0.110
ERROR	10511.66	36	291.99		
WITHIN SUBJECTS					
Trials	25516.60	8	3189.58	11.36	0.000
Environ by Trials	2897.74	16	181.11	0.65	0.846
ERROR	80841.88	288	280.70		
TOTAL	121138.86	350			

Summary table of ANOVA of number of squares entered by the three offspring groups over the nine trials on the first practice maze.

TRIAL	SEC	SC	IC
1	43.46	54.00	50.31
2	25.30	27.46	31.08
3	29.23	25.38	31.30
4	27.15	26.61	29.61
5	24.00	21.61	24.00
6	18.84	16.69	33.46
7	18.15	23.23	24.07
8	26.38	19.15	22.61
9	16.38	16.76	21.15

Mean number of squares entered for the three offspring groups, over the nine trials of Practice Maze A, day one of training.

SOURCE	S.O.S	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Environ	63288.55	2	31644.28	5.64	0.007
ERROR	201853.40	36	5607.04		
WITHIN SUBJECTS					
Trials	189016.98	8	23627.12	11.56	0.000
Environ by Trials	47518.01	16	2969.88	1.45	0.117
ERROR	588709.68	288	2044.13		
TOTAL	1090386.6	350			

Summary table of ANOVA of time to reach the goal box in the first practice maze, over the nine trials for the three offspring groups.

TRIAL	SEC	SC	IC
1	46.07	130.76	125.23
2	22.00	63.84	82.07
3	23.46	59.15	52.46
4	22.00	43.61	38.38
5	16.53	30.23	30.38
6	18.15	26.92	29.23
7	17.53	27.76	29.46
8	22.30	48.76	25.53
9	14.30	44.46	25.00

Mean time to reach the goal box for the three offspring groups over the nine trials on Practice Maze A, first training day.

MEANS	SEC SC IC	56.076 92.512 114.412
MS ERROR	29059.37	
D.F.	36	
N PER GROUP	78	
CRITICAL DIFFERENCE	r=2 r=3	q(0.05)=24.50 q(0.01)=29.58 q(0.05)=32.98 q(0.01)=39.34
COMPARISON	SEC vs IC SEC vs SC SC vs IC	p<0.01 p<0.01 N/S

Summary table of Newman Keuls performed on the time to reach goal box data.

HEBB-WILLIAMS: TESTING

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Environ	6.82	2	3.41	0.95	0.39756
ERROR	128.92	36	3.58		
TOTAL	135.74	38			

Summary table of ANOVA of total number of retraces (added for each animal over the six test mazes and eight trials per maze, for the three groups).

GROUP	MEAN
SEC	0.92
SC	1.85
IC	1.77

Means of total number of retraces over the six test mazes, for eight trials per mazes, for the three offspring groups.

SOURCE	S.O.S	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Environ	48.12	2	24.06	0.99	0.38476
ERROR	878.36	36	24.40		
WITHIN SUBJECTS					
Trials	1628.94	7	232.71	27.86	0.00000
Trials by Environ	84.23	14	6.02	0.72	0.75415
ERROR	2104.87	252	8.35		
Mazes	1189.21	5	237.84	18.33	0.00000
Mazes by Environ	154.89	10	15.49	1.19	0.29724
ERROR	2335.69	180	12.98		
Trials by Mazes	477.94	35	13.66	1.68	0.00859
Trials by Mazes by Environ	624.04	70	8.91	1.09	0.28282
ERROR	10271.2	1260	8.15		
TOTAL	19797.5	1871			

Summary table of ANOVA of number of errors made by the three offspring groups over the six test mazes with eight trials on each maze.

GROUP	MAZE	TRIAL 1	2	3	4	5	6	7	8
SEC	1	2.92	1.31	1.00	0.54	1.77	0.15	0.23	0.15
SEC	3	5.85	4.15	3.00	1.46	1.46	0.77	1.31	1.08
SEC	5	6.54	7.54	4.77	3.23	2.69	3.46	3.54	3.54
SEC	7	5.85	5.62	3.00	2.54	2.00	4.46	1.77	2.69
SEC	9	3.23	3.00	2.85	1.92	2.08	2.23	2.23	2.77
SEC	11	4.62	3.31	2.92	2.69	3.92	1.85	2.00	2.62
SC	1	4.00	1.62	0.77	0.85	0.15	0.69	0.77	1.69
SC	3	5.15	2.54	2.85	2.00	1.23	2.15	0.69	0.46
SC	5	5.31	5.69	3.77	3.31	2.62	4.31	2.69	1.92
SC	7	5.00	2.54	2.54	2.92	2.54	1.85	2.00	1.38
SC	9	4.62	2.54	3.23	1.54	1.69	2.46	2.23	1.23
SC	11	2.46	3.92	2.15	4.69	1.15	2.00	1.38	1.31
IC	1	3.77	1.77	0.54	1.08	0.62	0.69	1.23	1.08
IC	3	7.00	3.62	2.08	1.85	2.31	1.00	1.15	1.15
IC	5	6.85	4.62	3.38	3.92	3.00	3.08	2.23	2.08
IC	7	2.23	2.08	3.31	2.54	2.46	2.69	2.15	1.62
IC	9	3.62	4.77	2.92	3.69	3.23	2.31	3.08	2.85
IC	11	3.38	6.15	4.38	1.54	2.77	2.08	1.85	2.69

Mean number of errors for the three offspring groups, over the six test mazes with eight trials on each maze. (N=13 per group)

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Environ	9.96158	2	4.98079	0.64	0.53750
ERROR	279.962	36	7.77671		
Trials	102.162	7	14.5946	4.59	0.00018
Trials by Environ	35.0898	14	2.50641	0.79	0.68231
ERROR	800.936	252	3.17832		
Mazes	158.774	5	31.7548	6.20	0.00009
Mazes by Environ	19.4040	10	1.94040	0.38	0.95434
ERROR	921.634	180	5.12019		
Trials by Mazes	120.726	35	3.44931	1.06	0.37831
Trials by Mazes by Environ	231.647	70	3.30924	1.01	0.44611
ERROR	4109	1260	3.26126		
TOTAL	6789.55	1871			

Summary table of ANOVA of number of rears made by the three offspring groups over the six mazes, with eight trials on each maze.

GROUP	MAZE	TRIAL 1	2	3	4	5	6	7	8
SEC	1	0.54	0.38	0.08	0.23	1.38	0.08	0.15	0.00
SEC	3	1.38	0.85	0.69	0.31	0.31	0.00	0.15	0.15
SEC	5	1.77	2.31	1.62	0.85	0.46	0.54	1.08	0.46
SEC	7	0.69	0.85	0.46	0.38	0.08	0.85	0.15	0.62
SEC	9	0.46	1.23	1.38	0.46	0.54	0.69	1.23	1.23
SEC	11	1.54	1.54	1.62	0.23	1.23	0.38	1.38	2.31
SC	1	0.62	0.54	0.23	0.46	0.00	0.31	0.31	0.69
SC	3	1.15	0.85	0.15	0.69	0.31	0.77	0.31	0.08
SC	5	0.54	1.54	1.38	1.23	0.92	1.46	0.69	0.46
SC	7	1.15	0.38	0.85	0.54	1.23	0.46	0.31	0.46
SC	9	2.62	0.92	1.54	0.54	0.69	1.46	0.31	0.54
SC	11	0.31	1.23	1.00	1.77	0.54	0.54	0.46	0.85
IC	1	0.85	0.85	0.54	0.08	0.31	0.69	0.23	0.62
IC	3	1.92	0.62	0.54	0.85	0.85	0.38	0.15	0.23
IC	5	2.62	1.31	1.31	1.46	0.54	0.62	0.69	0.23
IC	7	0.38	1.00	0.92	0.38	0.62	0.38	1.00	0.69
IC	9	0.85	1.46	1.00	0.85	1.15	0.62	0.85	2.08
IC	11	1.23	3.92	1.69	0.69	1.46	0.62	0.54	1.38

Mean number of rears of the three offspring groups over the six test mazes, eight trials per maze. (N=13 per group)

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECT					
Environ	259.69	2	129.85	0.12	0.88550
ERROR	40235.4	36	1117.65		
WITHIN SUBJECTS					
Trials	66594.0	7	9513.44	20.31	0.00000
Trials by Environ	4573.43	14	326.67	0.70	0.77715
ERROR	118062.0	252	468.50		
Mazes	43705.3	5	8741.06	11.83	0.00000
Mazes by Environ	45507.1	10	4550.71	6.16	0.00000
ERROR	133035.0	180	39.09		
Trials by Mazes	19708.7	35	563.11	1.34	0.08862
Trials by Mazes by Environ	39963.7	70	570.91	1.36	0.02782
ERROR	528449.0	1260	419.40		
TOTAL	1.04009E6	1871			

Summary table of ANOVA of number of squares entered by the three offspring groups over the six test mazes with eight trials on each maze.

GROUP	MAZE	TRIAL 1	2	3	4	5	6	7	8
SEC	1	33.00	25.00	11.46	11.77	18.62	7.08	7.77	6.85
SEC	3	36.46	26.85	24.15	15.85	18.00	14.23	15.62	14.15
SEC	5	46.23	59.00	37.31	30.23	25.77	28.15	28.77	26.46
SEC	7	43.15	32.46	21.38	20.92	18.92	31.31	20.38	21.00
SEC	9	31.92	42.77	34.54	26.23	28.00	31.85	30.08	35.38
SEC	11	38.62	23.62	30.69	24.38	33.08	18.77	22.00	25.54
SC	1	39.23	21.00	22.23	20.46	15.69	20.92	12.62	11.23
SC	3	50.69	48.69	32.85	31.54	22.54	33.31	24.23	21.31
SC	5	38.85	22.77	25.23	20.69	22.92	18.62	17.69	16.31
SC	7	56.38	33.31	35.69	24.54	29.54	42.00	28.77	24.69
SC	9	28.69	38.77	25.00	45.31	19.31	23.54	21.92	19.38
SC	11	29.00	13.54	10.85	10.00	7.38	12.00	11.54	21.08
IC	1	54.62	33.85	23.77	21.46	18.77	20.15	15.69	18.54
IC	3	44.54	29.15	32.31	34.92	35.15	24.85	24.54	18.62
IC	5	28.00	25.31	26.38	21.85	21.08	21.77	32.62	25.00
IC	7	28.54	66.31	38.00	31.54	37.46	29.77	28.38	41.77
IC	9	32.23	33.15	26.62	18.46	23.23	21.92	17.92	18.54
IC	11	32.85	16.00	10.54	13.46	12.69	11.08	10.23	12.15

Mean number of squares entered by the three offspring groups over the six test mazes, eight trials on each maze. (N=13 per group)

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Environ	3471.11	2	1735.55	1.76	0.18426
ERROR	35424.7	36	984.02		
WITHIN SUBJECTS					
Trials	66012.9	7	9430.42	17.31	0.00000
Trials by Environ	29059.4	14	2075.67	3.81	0.00004
ERROR	137251.0	252	544.65		
Mazes	1602.89	5	320.58	0.98	0.43451
Mazes by Environ	3121.84	10	312.18	0.95	0.48867
ERROR	59090.0	180	328.28		
Trials by Mazes	7097.92	35	202.80	1.05	0.39006
Trials by Mazes by Environ	10473.0	70	149.61	0.77	0.91355
ERROR	243363.0	1260	193.15		
TOTAL	595967.0	1871			

Summary table of ANOVA of time taken by the three offspring groups to emerge from the start box, over the six test mazes with eight trials on each maze.

GROUP	MAZE	TRIAL 1	2	3	4	5	6	7	8
SEC	1	2.00	0.62	0.23	0.08	0.15	0.00	0.77	0.31
SEC	3	11.23	0.46	0.69	0.38	0.46	0.85	0.38	0.54
SEC	5	9.38	0.77	0.54	1.54	0.15	0.00	0.23	1.77
SEC	7	8.38	0.23	0.54	1.85	1.46	2.15	0.46	0.54
SEC	9	2.62	2.23	1.54	5.31	13.46	17.31	1.31	1.38
SEC	11	4.15	0.62	5.38	0.31	1.08	0.92	0.38	0.62
SC	1	14.23	1.46	0.23	0.85	0.23	0.08	0.31	0.54
SC	3	14.08	1.92	0.62	0.31	0.23	0.31	0.54	1.00
SC	5	16.15	0.77	0.31	0.23	0.31	1.15	0.23	1.38
SC	7	17.92	0.77	2.38	0.46	0.77	0.46	0.23	0.46
SC	9	15.31	5.77	1.38	0.31	0.62	2.62	0.38	0.77
SC	11	23.77	2.46	1.15	2.08	0.92	1.85	1.08	0.54
IC	1	18.31	1.54	1.08	0.15	0.54	0.77	0.38	0.54
IC	3	58.62	6.08	1.38	0.54	0.85	0.31	0.31	1.62
IC	5	42.23	13.62	2.54	2.92	0.54	0.23	0.77	0.62
IC	7	26.92	1.00	0.92	0.15	1.31	0.62	0.77	0.15
IC	9	31.00	2.77	2.00	0.46	0.46	0.23	0.38	0.85
IC	11	29.00	1.62	0.46	1.08	0.38	0.38	0.46	0.46

Mean latency (in seconds) to emerge from the start box for the three offspring groups, over the six test mazes, eight trials per maze. (N=13 per group)

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Environ	22558.60	2	711279.30	2.68	0.082
ERROR	151490.58	36	4208.07		
WITHIN SUBJECTS					
Trials	474727.62	7	67818.23	34.05	0.000
Trials by Environ	99939.13	14	7138.51	3.58	0.000
ERROR	501961.06	252	1991.91		
Mazes	8229.00	5	1645.80	2.93	0.014
Mazes by Environ	6830.03	10	683.00	1.22	0.284
ERROR	101132.49	180	561.85		
Trials by Mazes	46591.34	35	1331.18	3.14	0.000
Trials by Mazes by Envir	43378.49	70	619.69	1.46	0.009
ERROR	533484.48	1260	423.40		
TOTAL	1990322.82	1871			

Summary table of ANOVA of subjects time to reach the goal box for the three offspring groups over the six mazes, with eight trials on each maze.

GROUP	MAZE	TRIAL 1	2	3	4	5	6	7	8
SEC	1	23.84	3.69	3.38	3.46	3.15	2.30	2.23	2.15
SEC	3	37.46	9.23	5.69	4.30	4.07	2.23	3.23	3.92
SEC	5	36.69	15.38	11.53	9.07	6.76	8.00	6.30	6.15
SEC	7	18.46	12.84	5.30	10.30	17.61	23.76	4.23	6.69
SEC	9	24.23	10.76	14.15	6.07	7.07	7.46	9.15	8.15
SEC	11	27.84	10.69	8.69	5.23	9.30	5.46	7.23	7.92
SC	1	65.00	7.30	3.23	3.07	1.69	2.38	2.15	3.23
SC	3	55.07	9.61	7.00	7.07	4.30	9.76	2.84	3.92
SC	5	59.15	22.53	12.07	12.53	7.38	9.84	6.53	6.15
SC	7	43.23	26.07	8.92	7.15	7.38	7.69	3.61	4.76
SC	9	72.61	19.53	14.23	10.38	14.53	15.15	10.46	6.92
SC	11	42.23	18.84	7.07	15.46	5.61	7.23	4.46	4.61
IC	1	109.84	12.92	5.23	4.07	3.76	4.61	3.84	5.69
IC	3	98.53	22.69	7.23	10.38	5.53	4.23	3.46	3.46
IC	5	116.30	14.61	11.23	10.38	10.07	8.92	6.00	4.53
IC	7	56.15	8.38	16.61	6.53	5.84	4.61	4.00	4.38
IC	9	75.15	21.53	10.07	11.46	12.46	6.61	8.92	12.76
IC	11	41.23	43.07	17.00	5.61	10.15	6.15	6.38	8.00

Mean time to arrive at goal box for the three offspring groups over the six test mazes, eight trials per maze (timer started when animal placed in start box).
(N=13 per group)

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Environ	18382.23	2	9191.11	1.55	0.226
ERROR	213683.46	36	5935.65		
WITHIN SUBJECTS					
Trials	585131.56	7	83590.22	31.01	0.00000
Trials by Environ	89175.80	14	6369.70	2.36	0.004
ERROR	679279.10	252	2695.55		
Mazes	7219.02	5	1443.80	1.99	0.083
Mazes by Environ	6840.55	10	684.06	0.94	0.497
ERROR	13862.13	180	727.01		
Trials by Mazes	52610.08	35	1503.15	2.58	0.00001
Trials by Mazes by Environ	46437.68	70	663.40	1.14	0.206
ERROR	733276.53	1260	581.97		
TOTAL	2728398.1	1871			

Summary table of ANOVA of total time taken by the three offspring groups in the Hebb-Williams maze, over the six test mazes, with eight trials on each maze.

GROUP	MAZE	TRIAL 1	2	3	4	5	6	7	8
SEC	1	40.14	4.92	4.38	4.53	8.38	2.53	3.30	3.46
SEC	3	45.69	35.38	6.69	5.30	5.07	3.53	7.76	4.84
SEC	5	38.61	20.46	12.61	11.53	7.76	8.93	6.46	7.00
SEC	7	24.07	13.92	8.23	13.07	18.61	26.07	5.07	7.76
SEC	9	28.84	11.69	15.15	7.07	7.92	8.30	10.07	9.23
SEC	11	32.07	11.46	9.30	6.00	10.15	6.07	7.84	8.76
SC	1	71.07	8.69	4.46	4.23	2.69	5.23	4.84	13.00
SC	3	73.07	10.84	8.15	8.15	5.38	10.92	3.92	4.92
SC	5	72.53	23.84	13.30	16.53	8.38	10.84	7.53	7.38
SC	7	54.69	27.30	11.07	8.53	8.38	8.84	4.76	5.84
SC	9	79.07	23.92	15.61	12.38	15.84	18.00	11.46	8.07
SC	11	46.76	19.92	8.00	16.38	6.15	7.84	6.30	6.07
IC	1	112.23	16.15	6.23	6.23	6.00	9.69	4.84	7.15
IC	3	91.15	24.53	8.69	11.53	8.38	5.38	4.46	4.53
IC	5	126.15	20.53	12.23	12.30	11.07	9.15	5.46	5.46
IC	7	64.92	9.53	17.76	7.38	8.84	5.46	7.53	5.23
IC	9	79.92	21.92	10.92	12.38	13.30	7.53	9.69	13.61
IC	11	43.38	44.00	17.84	6.07	11.07	6.69	7.23	9.46

Mean total time taken to eat all food (timer started when animal placed in start box) for the three offspring groups, over the six test mazes, eight trials per maze.
(N=13 per group)

EXPERIMENT TWO

SKINNER BOX: LITTERSIZE

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Environ	127.82	2	63.91	12.76	0.00011
ERROR	270.42	54	5.01		
TOTAL	398.25	56			

Summary table of ANOVA of littersize that the three offspring groups employed in the Skinner box experiment came from.

GROUP	MEANS
SEC	9.16
SC	12.79
IC	10.53

Means of the three offspring groups' littersizes of the animals used in the Skinner box experiment. (N=19 per group)

SOURCE	SCORE	PROBABILITY
BARTLETT-BOX F	2.05358	0.129

Test for homogeneity of variance between the three offspring groups' scores totalled over the fourteen days of testing, prior to carrying out the ANCOVA detailed below.

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN GROUPS					
Regression	3434.45	1	3434.45	0.10	0.751
Environ	735387.37	2	367693.68	10.92	0.000
ERROR	1784373.05	53	33667.42		
WITHIN SUBJECTS					
Days	4958840.36	13	381449.26	118.52	0.000
Days by Environ	391880.00	26	15072.31	4.68	0.000
ERROR	2259280.29	702	3218.35		
TOTAL					

Summary table of ANCOVA carried out on the number of bar presses per animal over the fourteen days of Skinner box trials, with each subjects' littersize as the co-variate, for the three offspring groups (for means, see chapter seven Figure 7:7).

APPENDIX: CHAPTER EIGHT

LITTERSIZE

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Drug Dose	21.49	4	5.37	0.35	0.84516
Environ	16.96	2	8.48	0.55	0.58468
Drug Dose by Environ	50.38	8	6.30	0.41	0.91229
ERROR	1156.17	75	15.42		
TOTAL	1244.99	89			

Summary table of ANOVA of the littersize of each subject employed in chapter eight subdivided by drug dose and the three offspring groups.

GROUP	DOSE A	DOSE B	DOSE C	DOSE D	DOSE E
SEC	8.50	11.17	11.33	8.17	9.67
SC	10.83	10.17	10.17	10.00	9.17
IC	9.67	11.17	10.67	11.00	11.50

Means of the offspring groups' littersizes, N=6 per group of the fifteen groups of animals. (three offspring experimental backgrounds, five drug doses; N=6 per group)

SKINNER BOX

LAST PRE-DRUG DAY: DAY 15

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Environ	13750.289	2	6875.144	0.526	0.593
Drug Dose	46542.289	4	11635.572	0.891	0.474
Environ by Drug Dose	113345.711	8	14168.214	1.085	0.383
ERROR	979539.00	75	13060.520		
TOTAL	1153177.289	89	12957.048		

Summary table of ANOVA of number of bar presses on day fifteen of training, that is the last pre-drug day.

GROUP	DOSE A	DOSE B	DOSE C	DOSE D	DOSE E
SEC	191.33	249.50	158.00	164.67	165.67
SC	155.83	173.67	199.00	259.17	266.00
IC	125.33	148.83	207.67	259.50	175.50

Mean number of bar presses of the three offspring groups (divided up by future dose group) on the last day of Skinner box training prior to drug administration.

INITIAL ELEVEN DAYS OF SKINNER BOX TRAINING

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Environ	12999.59	2	6499.79	0.27	0.762
Drug Dose	78734.31	4	19683.58	0.83	0.512
Environ by Drug Dose	161848.04	8	20231.00	0.85	0.562
ERROR	1783614.59	75	23781.53		
WITHIN SUBJECTS					
Trials	3688751.10	10	368875.11	141.47	0.000
Trials by Environ	27745.65	20	1387.28	0.53	0.954
Trials by Drug Dose	89946.47	40	2248.66	0.86	0.712
Trials by Environ by Dose	201565.72	80	2519.57	0.97	0.562
ERROR	1954201.24	750	2605.60		
TOTAL	7919406.71	989			

Summary table of ANOVA of number of bar presses over the initial eleven days of Skinner box training prior to administration of drug doses, for the three offspring groups. (Subdivided into future drug groups N=6 per group)

DAY	SCHEDULE	SEC	SC	IC
1	CRF	7.53	8.36	7.76
2	CRF	10.30	9.40	9.86
3	CRF	12.40	14.00	14.03
4	CRF	18.90	21.00	17.63
5	CRF	29.93	30.33	25.80
8	FR3	58.56	60.63	62.86
9	FR3	79.10	75.46	75.06
10	FR6	102.70	101.96	92.66
11	FR6	126.60	134.13	111.83
12	FR6	149.20	171.33	139.23
15	FR6	185.83	210.73	183.36

Mean number of bar presses for the three offspring groups over the first eleven days of Skinner box training, prior to administration of the drug doses.

LAST THREE DAYS OF TESTING

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Environ	76833.54	2	38416.77	0.63	0.535
Drug Dose	702447.43	4	175611.86	2.89	0.028
Environ by Drug Dose	77636.90	8	9704.61	0.16	0.995
ERROR	4562534.11	75	60833.79		
WITHIN SUBJECTS					
Trials	50132.81	2	25066.40	3.41	0.036
Trials by Environ	22880.57	4	5720.14	0.78	0.541
Trials by Dose	89608.30	8	11201.04	1.52	0.153
Trials by Environ by Dose	74640.10	16	4665.01	0.63	0.852
ERROR	1102076.22	150	7347.17		
TOTAL	6758790.08	269			

Summary table of ANOVA of number of bar presses over the last three days of Skinner box testing, for the three offspring groups' subdivided into their five drug dose groups.

GROUP	DAY 16	DAY 17	DAY 18
SEC DOSE A	181.16	210.83	181.16
SEC DOSE B	287.00	274.33	337.16
SEC DOSE C	237.33	216.83	238.50
SEC DOSE D	242.83	168.66	213.66
SEC DOSE E	183.66	145.83	150.00
SC DOSE A	206.00	194.00	215.16
SC DOSE B	328.50	301.33	302.00
SC DOSE C	256.33	244.66	205.33
SC DOSE D	294.50	224.83	198.83
SC DOSE E	209.50	112.00	108.66
IC DOSE A	164.66	181.50	229.16
IC DOSE B	295.16	379.83	270.66
IC DOSE C	355.50	304.00	311.83
IC DOSE D	302.66	255.66	256.83
IC DOSE E	254.33	152.33	145.50

Mean number of bar presses for the three offspring groups, subdivided by drug dose groups, over the three days of amphetamine administration. (N=6 per group)

OPEN FIELD

NUMBER OF LINES CROSSED

SOURCE	S.O.S	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Environ	28070.2	2	14035.1	0.98	0.38206
Drug Dose	71826.3	4	17956.6	1.25	0.29534
Environ by Drug Dose	59772.7	8	7471.59	0.52	0.83745
ERROR	1.07468E6	75	14329.0		
WITHIN SUBJECTS					
Days	32415.4	4	8103.85	10.90	0.00000
Days by Environ	2876.80	8	359.60	0.48	0.86787
Days by Drug Dose	13642.9	16	852.68	1.15	0.31024
Days by Environ By Dose	18758.8	32	586.21	0.79	0.78868
RESIDUAL	222979.0	300	743.26		
TOTAL	1.52502E6	449			

Summary table of ANOVA of number of lines crossed over the five days of testing for the three offspring groups, subdivided into their five drug dose groups.

GROUP	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
SEC DOSE A	111.33	129.67	117.83	101.17	104.67
SEC DOSE B	103.50	169.67	128.17	117.33	125.50
SEC DOSE C	110.50	194.83	138.50	116.67	133.33
SEC DOSE D	124.17	187.00	125.83	131.83	132.67
SEC DOSE E	112.67	202.17	142.00	122.00	135.33
SC DOSE A	97.83	123.83	101.50	101.50	92.33
SC DOSE B	105.33	153.00	119.83	111.50	95.17
SC DOSE C	115.83	157.67	151.50	109.50	90.83
SC DOSE D	130.83	153.50	140.00	116.67	94.83
SC DOSE E	115.83	137.83	151.50	134.00	94.67
IC DOSE A	107.67	102.50	100.33	100.33	112.00
IC DOSE B	117.17	116.17	102.33	120.33	98.83
IC DOSE C	130.67	113.67	128.17	115.50	95.50
IC DOSE D	119.17	107.67	113.50	130.33	104.17
IC DOSE E	131.50	115.33	139.00	119.00	103.83

Mean number of lines crossed for the three offspring groups over the five days of open field testing, subdivided by their drug dose groups. (N=6 per group)

NUMBER OF REARS

SOURCE	S.O.S.	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Environ	494.49	2	247.24	1.12	0.33325
Drug Dose	2641.46	4	660.36	2.98	0.02390
Environ by Drug Dose	1047.18	8	130.90	0.59	0.78335
ERROR	16606.3	75	221.42		
WITHIN SUBJECTS					
Days	807.06	4	201.76	9.70	0.00001
Days by Environ	175.85	8	21.98	1.06	0.39341
Days by Drug Dose	505.03	16	31.56	1.52	0.09167
Days by Environ by Dose	556.93	32	17.40	0.84	0.72224
ERROR	6238.26	300	20.79		
TOTAL	29072.6	449			

Summary table of ANOVA of number of rears made by the three offspring groups subdivided into their five drug groups, over the five days of open field testing.

GROUP	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
SEC DOSE A	18.33	13.67	9.83	8.50	7.17
SEC DOSE B	8.17	12.67	9.00	10.00	6.50
SEC DOSE C	8.00	10.00	7.50	9.50	6.67
SEC DOSE D	9.67	9.83	6.67	6.00	6.50
SEC DOSE E	10.17	11.17	6.33	5.57	5.83
SC DOSE A	16.00	16.17	10.83	7.33	10.17
SC DOSE B	10.33	18.17	9.33	5.83	7.17
SC DOSE C	13.17	14.17	8.83	7.00	7.50
SC DOSE D	13.67	11.83	11.17	3.17	6.83
SC DOSE E	10.33	20.50	12.33	5.33	8.33
IC DOSE A	17.33	12.17	13.00	12.50	4.33
IC DOSE B	11.33	6.83	8.83	4.67	2.17
IC DOSE C	10.50	6.00	10.50	5.67	5.00
IC DOSE D	11.33	6.00	6.67	5.67	4.67
IC DOSE E	12.50	7.17	8.33	3.67	5.67

Mean number of rears over the five days of open field testing for the three offspring groups, subdivided into their five drug groups. (N=6 per group)

NUMBER OF DEFECATIONS

SOURCE	S.O.S	D.F.	M.S.	F RATIO	PROBABILITY
BETWEEN SUBJECTS					
Environ	1.33333	2	0.66667	0.65	0.52997
Drug Dose	3.43554	4	0.85889	0.84	0.50814
Environ by Drug Dose	8.71111	8	1.08889	1.06	0.39990
ERROR	77.0001	75	1.02667		
WITHIN SUBJECTS					
Days	2.01333	4	0.50333	2.53	0.03993
Days by Environ	1.000000	8	0.12500	0.63	0.75529
Days by Drug Dose	3.40891	16	0.21306	1.07	0.38203
Days by Environ by Dose	5.51112	32	0.17222	0.87	0.67940
ERROR	59.6673	300	0.19889		
TOTAL	162.081	449			

Summary table of ANOVA of number of defecations made by the three offspring groups (subdivided into their five drug groups) over the five days of open field testing.

GROUP	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
SEC DOSE A	0.17	0.00	0.50	0.50	0.17
SEC DOSE B	0.83	0.00	0.00	0.17	0.00
SEC DOSE C	0.00	0.00	0.00	0.00	0.00
SEC DOSE D	0.00	0.00	0.00	0.00	0.00
SEC DOSE E	0.00	0.00	0.00	0.00	0.00
SC DOSE A	0.00	0.00	0.00	0.17	0.00
SC DOSE B	0.00	0.50	0.00	0.00	0.00
SC DOSE C	0.00	0.00	0.00	0.00	0.00
SC DOSE D	0.00	0.00	0.00	0.00	0.00
SC DOSE E	0.00	0.00	0.00	0.00	0.00
IC DOSE A	0.00	0.50	0.00	0.00	0.50
IC DOSE B	0.00	1.17	0.00	0.00	0.00
IC DOSE C	0.00	0.67	0.00	0.00	0.00
IC DOSE D	0.00	1.00	0.00	0.00	0.00
IC DOSE E	0.00	0.17	0.00	0.00	0.00

Mean number of defecations by the three offspring groups, subdivided by drug dose group, over the five days of open field testing. (N=6 per group)