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**The effects of early experience on cognitive functioning in the
rat**

Wilson, Lynn Allison, M.A.

The University of Arizona, 1989

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THE EFFECTS OF EARLY EXPERIENCE ON
COGNITIVE FUNCTIONING IN THE RAT

by

Lynn Allison Wilson

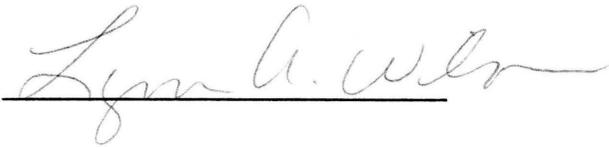
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DEPARTMENT OF PSYCHOLOGY
In Partial Fulfillment of the Requirements
For the Degree of
MASTER OF ARTS
In the Graduate College
THE UNIVERSITY OF ARIZONA

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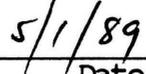
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ABSTRACT

Forty-eight rat pups were handled and isolated from postnatal days 3 through 13 in order to determine whether this manipulation would alter the postnatal development of the hippocampus. Half of these animals were then reared in enriched environments from weaning until maturity to determine whether enrichment would ameliorate the expected deficits in learning ability. Beginning at 90 days of age, all animals were tested on a T-maze, rotating bar and both place and cued versions of a water maze task.

The study failed to find gross deficits in learning as a result of the handling/isolation procedure, although emotional differences between groups were evident, as were sex differences. Apparently more questions have been raised than answered by this study, and possible directions for future research are discussed.

INTRODUCTION

Because the mammalian brain is neither fully developed nor completely functional at birth, environmental events occurring early in life may have profound and lasting effects on structure and function. The visual system for example undergoes significant postnatal maturation of the retina, in myelination of the optic nerves and in the geniculocortical projections that form ocular dominance columns (Bronson, 1974; Hickey and Peduzzi, 1987). Altering the visual environment during the time that ocular dominance columns are forming has been shown to produce long-term effects on the organization of these columns (Wiesel and Hubel, 1965).

Another area that is susceptible to alterations in final form is the hippocampus, this due to its late development. The majority of granule cells in the dentate gyrus are generated after birth in the mouse (Angevine, 1965), rat (Altman and Das, 1965; Schlessinger, Cowan and Gottleib, 1975; Bayer, 1980), and primate (Rakic and Nowanowski, 1981; Rakic, 1985). Although the proliferation of these cells peaks early in life; at 4 days in the mouse (Angevine, 1965) and about 2 months in the human (Kretschmann et al., 1986), granule cells continue to be produced throughout life (Eckenhoff and Rakic, 1988).

Hippocampal synaptogenesis also occurs primarily postnatally in the rat (Crain, Cotman, Taylor and Lynch, 1973), not reaching adult levels until 25 days of age. Finally, the glucocorticoid receptor system known to be highly concentrated in the adult hippocampus is

engaged in a phase of rapid development during the early postnatal period. Recent work indicates the presence of two distinct receptor types (Rosenfeld, Sutanto, Levine and DeKloet, 1988; Sarrieau, Sharma and Meaney, 1988), each with a unique developmental pattern (Rosenfeld et al., 1988). The mineralocorticoid (or Type I) receptors, which in the central nervous system are confined primarily to the septohippocampal system and pituitary are undetectable during the first few days following birth. These receptors can be measured at adult levels by 8 days of age in the rat, and undergo very little change after that time. The classical glucocorticoid (or Type II) receptors, which are widely distributed throughout the brain, are present at birth. The developmental changes of these receptors are primarily related to affinity and concentration. In adult rats, Type I receptors bind corticosterone (CORT) with high affinity, while Type II receptors bind CORT with low to moderate affinity. During the ontogenetic absence of Type I receptors, Type II receptors bind CORT with high affinity. This high Type II affinity begins to decrease when Type I receptors "turn on" somewhere between 4 and 8 days and reaches adult levels by day 8 (Rosenfeld, et al, 1988). Concentration changes are also evident in Type II receptors. At 2-4 days immunoreactive staining shows the receptors distributed in the pyramidal cells of all hippocampal regions, CA1-CA4, and in the granule cells of the dentate gyrus. Immunoreactive staining decreases in all areas, and completely disappears from CA3 and CA4 by day 12. Evidence of increased staining is seen in CA1 and CA2

and the dentate gyrus at day 16, and reaches adult levels by day 20 (Rosenfeld, Van Eekelen, Levine and DeKloet, 1988). The results of this recent work have led both Rosenfeld and her colleagues, and Sarrieau's group to suggest that developmental plasticity of the classical glucocorticoid receptors may be responsible for the effects obtained through manipulations of the early environment.

Thus, while most central nervous system development occurs in the relatively protected prenatal environment, a significant amount of growth remains to be accomplished after birth. The postnatal environment is far less stable and predictable than that found prenatally, leaving young animals vulnerable to conditions unfavorable to optimal development.

Hippocampal Function

Studies with animals have shown that the hippocampus plays a role in some, but not all, forms of learning and memory. More specifically, the hippocampus appears to be necessary for acquiring and retaining information about where and in what context things happen. Rats without intact hippocampi are selectively impaired on spatial tasks such as the eight-arm radial maze (Barnes, 1988; Becker, Walker and Olton, 1980), and on place tasks in a water maze (Morris, Garrud, Rawlins and O'Keefe, 1982). A common feature of these tasks is that the animal must carry information from one trial to the next in order to successfully solve the problem. Hippocampal animals also show less evidence of spontaneous alternation (Kirby, Stein, Kimble and Kimble, 1967) and generally less exploratory behavior (Leaton, 1965). These characteristics in

normal animals may represent the acquisition of information about where and in what context events occur in the environment (O'Keefe and Nadel, 1978), which as suggested above may represent an important domain of hippocampal function.

In addition to its role in cognition, the hippocampus also plays a role in emotional processes. The primary physiological response of the body to stress is the activation of the adrenal-cortical axis, resulting in the release of glucocorticoids into the system. Thus, the body is prepared to deal with potential environmental threats. Long term maintenance of this state can have negative effects on various systems however, and a return to homeostasis normally follows termination of the stressor (Munck, Guyre and Holbrook, 1984). The hippocampus is thought to play a role in closing the feedback loop, thus halting further release of glucocorticoids into the system (Sapolsky and Meaney, 1986). The hippocampus has one of the highest concentrations of corticosterone binding in the rat brain (Sarrieau, Philibert and Rostene, 1984), and mineralocorticoid (Type I) receptors are found almost exclusively in the septohippocampal region (Rosenfeld, et al., 1988, Sarrieau, et al., 1988). The activation of these sites may well be one of the mechanisms responsible for terminating the stress response.

In addition to the proposed role in the termination of the stress response, the hippocampal glucocorticoid receptors have been implicated in behavioral phenomena. Micco and McEwen (1980) found that corticosterone, but not dexamethasone, normalized appetitive

extinction in adrenalectomized rats. Although studies to date have not clearly delineated the behavioral effects of glucocorticoid receptor alterations in the hippocampus, current knowledge indicates that this is an area worthy of future effort.

Early Experience

A variety of early experiences has been shown to alter behavior, and to produce morphological changes in animals. Three of the most robust manipulations are handling, isolation and enrichment. Each will be further discussed here.

Handling

Young rat and mice pups that have been handled prior to weaning have generally been shown to be less emotional as adults, demonstrate increased learning capacities, and to exhibit neural differences throughout life (see Daly, 1973 for a review).

Emotionality has usually been measured by activity in an open field and by defecation, with the interpretation that less emotional animals defecate less and move around more (Daly, 1973). Generally, handled animals defecate less (Levine, 1959; Denenberg, Rosenberg, Haltmeyer and Whimbey, 1969; Denenberg and Morton, 1962) and are more active than nonhandled controls (DuPreez, 1964; Denenberg, et al., 1969). In addition to this behavioral evidence of reduced emotionality, it has been found that handled rats display reduced pituitary-adrenal response when subjected to cold stress (5 degrees C.) for 90 minutes (Levine and Lewis, 1959; Levine, 1962), and that handled rats have more glucocorticoid receptors in the hippocampus (Meaney, Aitken, Bodnoff, Iny,

Tatarewicz and Sapolsky, 1985).

Although handling in infancy appears to alter emotional responsivity, the results of studies to determine differential learning abilities are equivocal. Avoidance and tasks involving noxious stimuli show handled animals to be superior to nonhandled animals (Chevalier and Levine, 1955; Du Preez, 1964). A simple maze running task found no difference in running speed between handled and nonhandled animals (Spence and Maher, 1962). Wong and Jamieson (1968) found facilitory effects of handling on discrimination and reversal learning, while Wong (1966) found no effects of handling on acquisition or extinction in a T-maze. In a study using mice, Smith (1967) found handling to improve performance on brightness and pattern discrimination tasks, but to impair performance on a spatial task.

These findings suggest that some, but not all forms of learning are affected by handling. As many authors have noted the performance of animals on learning tasks is affected by their emotional reaction to the test situation (Daly, 1973; Smith, 1967; Chevalier and Levine, 1955; Spence and Maher, 1962). As handled animals react to novel situations with more willingness to explore, it might be expected that tasks related to movement through the environment would be most affected by handling. Surprisingly this is not the case. Tasks enhanced by handling include avoidance learning, those involving noxious stimuli and several varieties of discrimination. Running speed on a simple runway and acquisition and reversal of a T-maze showed no effect of handling, and spatial

learning was impaired by handling. Thus these results suggest an effect of handling on learning, but the nature of the effect has not been clearly defined.

The histological assessment of the effects of handling on neural development presents a rather more reliable picture than that of learning. There appears to be an increase in glial cell numbers in the anterior commissure and indusium griseum, as well as an increased number of myelinated axons in the anterior commissure of handled animals at 6 months of age (Sturrock, Smart and Tricklebank, 1983). Brain weights of handled animals are lower than nonhandled controls, as are areal measurements of the cerebellum, neocortex and hippocampus. Cell proliferation in these areas is at first slower in handled animals, but then increases to exceed that found in nonhandled animals (Altman, Das and Anderson, 1968). Both of these studies suggest that there is an initial retardation of brain growth, followed by a prolongation of neural development. The result is differences in adult neural structure which may account for functional differences observed in adult animals.

Another proposed mechanism for the effects of handling in infancy is differential development of the glucocorticoid receptor system. Dexamethasone binding has been found to be higher in the hippocampus and frontal cortex of handled animals (Meaney, Aitken, Bodnoff, Iny, Tatarewicz and Sapolsky, 1985). The classic glucocorticoid (or Type II) receptor binds with high affinity to dexamethasone, while the mineralocorticoid receptors bind with only

moderate affinity, leading to the conclusion by the authors of this study that these are the receptors affected by handling.

Interestingly, while this receptor is found throughout the brain, the increase in receptor numbers due to handling was limited to the hippocampus and frontal cortex.

Thus, although the experience of handling during infancy may produce lasting changes in the structure and function of the brain, it is still unclear exactly what these changes may be and which feature of the environment may be responsible for their mediation.

Isolation

Although the effects of handling on neural development have frequently been attributed to stress of the infant (cf. Altman et al, 1968; Meaney et al, 1985), studies that have sought to provide evidence of increased levels of glucocorticoids in stressed infants have found little evidence for this. Events that normally produce elevations of corticosterone in adult rats, such as surgery, ether, handling and thermal disruption, fail to have the same effect on infants (Sapolsky and Meaney, 1986). Thus it would appear difficult to elicit a pituitary-adrenal response in rats during the first two weeks of life. This is important because when an increase in circulating levels of glucocorticoids is induced by the administration of cortisol, corticosterone or dexamethasone, cells that are undergoing mitosis slow or halt division (Bohn, 1985). One manipulation that has been shown capable of increasing the normally low levels of corticosterone in rat pups is isolation from the dam (Stanton, Wallstrom and Levine, 1987). Following a period

of at least 8 hours of separation, rat pups show a significantly elevated level of corticosterone when exposed to novelty stress (Stanton and Levine, 1989; Stanton, Wallstrom and Levine, 1987). The long range effects of this manipulation on neural organization are not clear, but at least two possibilities exist. First, there may be a reduced number of late developing cells. Second, there may be an alteration in the number or concentration of glucocorticoid receptors.

Studies to date which have explored the behavioral effects of isolation in infancy have concentrated on social and emotional changes. Harlow's classic studies of monkeys reared in isolation indicate that these animals have severe social and emotional deficits in adulthood (Harlow and Zimmerman, 1959; Hinde and Spencer-Booth, 1971). Dogs have also been observed to display aberrant emotional behavior following severe sensory deprivation during development (Melzack and Thompson, 1956). Assessment of learning abilities in socially isolated monkeys demonstrated no deficits in general learning ability (Harlow, Harlow, Schlitz and Mohr, 1971). However, it should be noted that the tasks used by Harlow et al; discrimination, delayed response and oddity learning set, are not known to be hippocampally mediated and therefore would not be expected to be deficient if interference with development of the hippocampus is the major effect of isolation in animals. Far less is known of cognitive, specifically hippocampal function in rats that have been isolated during development.

Enriched Environment

As indicated above, handling and isolation of young animals probably exert their effects on developmental events occurring in the postnatal period, prior to weaning. A wide body of literature indicate that neural plasticity is not limited to this period, and many experiential events continue to influence neural organization and function. Time spent in enriched environments after weaning has effects on neural growth and may also affect learning ability.

Placing animals into environments that provide the opportunity for social and object interactions has been shown to increase various neural parameters relative to animals living in isolated or standard conditions. Rats reared in enriched environments consistently show heavier brains, increased cortical depth and increased dendritic branching (see Diamond, 1988; and Rosenzweig, 1971, for reviews). Whether these parameters are correlated with increases in learning ability is far less clear. At first glance, the data appear chaotic. Enriched animals appear to excel on some tasks, but not on others, while deprived animals outperform enriched subjects in some studies, and yet other studies find no differences between groups. These results suggest that enrichment does not increase the general cognitive or intellectual capacity of subjects per se, but rather exerts its effects on the response of the animals to the particular test situation. Bennett and Rosenzweig (1979) suggest that it is the complexity of the task that determines whether enrichment will have an effect. The Hebb-Williams maze generally distinguishes between groups, as does the

Lashley III maze, whereas a Y-maze, T-maze and sensory discrimination tasks do not. The criterion for determining complexity is not specified however, and it remains unclear that complexity can be quantitatively identified. Thus it appears that task complexity should either be better defined or broken into smaller and more easily defined components. Wilson, Warren and Abbott (1965) suggest that the superior performance in the Hebb-Williams maze may be due to the increased experience of enriched animals in exploring environments. A later study however found no difference in exploration as a result of enriched vs. restricted rearing environments (Engellenner, Goodlett, Burright and Donovick, 1982). Another explanation put forth to explain this difference in learning is an increase in fear-like responses seen in isolated or restricted animals (Myers and Fox, 1963). This explanation is consistent with that proposed by Harlow et al (1971), in which it was asserted that no learning differences exist between monkeys reared in various states of deprivation and enrichment. According to Harlow, once the emotional demands of the task have been considered (no painful or emotionally disturbing events should be used), and all animals have been adequately adapted to the test situation, there are no learning differences. It should be noted that the adaptation process as defined by Harlow and associates may constitute an environmental condition in itself, as evidenced by the following quote: "Fortunately these monkeys finally adapted after a thousand trials and made exemplary scores on subsequent multiple delay problems. Had we run an "abbreviated" 600-trial

test, we would have concluded that deprived environments produced "idiotcy". From these results it may be concluded that there is an interaction between emotional reactivity and the results obtained on learning tasks. However, it should also be noted that learning never occurs in the absence of an emotional state, and while emotions may be an important consideration in research on learning, it is not a separable entity. Perhaps instead of attempting to control the emotional state of the animal, it may be more productive to ask how this state affects learning.

Thus, handling, isolation and enrichment all appear to have some effect on emotional reactivity and some forms of learning; both of which have been linked to the hippocampus. These behavioral effects have been shown to be accompanied by neural changes in the hippocampus. Taken together these findings suggest that early experience can alter the development and later functioning of the hippocampus. In the present study rats were subjected to a handling/isolation procedure during the first two weeks of life that has been used to produce deficits in tasks known to be hippocampally mediated (Wilson, Willner, Kurz and Nadel, 1986; Nadel and Willner, in press). Following weaning, animals were reared in either standard laboratory conditions or placed into enriched environments, in an attempt to ameliorate the deficits due to the neonatal manipulation. It was expected that the handled/isolated animals would be impaired relative to control animals on tasks where they were required to acquire and retain information about places in the environment (place version of the

water tank and T-maze). A nonspatial task (cue version of the water tank) was included to rule out differences in general learning ability, and a motor task was used to insure that there were no differences in physical abilities that might account for performance differences in the water tank. A second question asked by this study was whether the expected deficit would be ameliorated by environmental enrichment. It was expected that handled/isolated animals reared in enriched conditions would perform similarly to control animals if this were the case.

METHOD

Subjects

Subjects for this study were 64 Long-Evans hooded rats bred in the animal laboratory at the University of Arizona. Eight litters were used. The litters were randomly assigned to a condition and culled to 8 pups (4 male and 4 female) on postnatal day one (PNI), with the day of birth designated PNO. All animals were maintained on a 12/12 hour light/dark cycle (lights on 2200 hrs) and had standard lab chow and tap water freely available throughout the experiment.

Handling and Isolation - Procedure

Four litters were randomly assigned to the handled/isolate (experimental) condition, the remaining four litters served as controls. On postnatal days 3 through 13 the dam was removed from the nest and placed into a holding cage. Each experimental litter was then removed from its nest and placed into a small cardboard compartment and gently shaken for 30 seconds followed by a 30

second rest. This sequence was repeated 5 times, after which the litters and then the dams were returned to the home nest. On PN days 9, 11 and 13, the litters were subjected to isolation rather than handling. The dam and then the pups were removed from the nest just prior to the onset of the dark cycle and the pups were placed on a nest in another room. The dam was returned to the home cage for the duration of the isolation period. The isolation nests consisted of a 3-inch layer of sawdust bedding placed in a 10-inch diameter plastic pail. This nest was elevated above a heating pad which maintained the surface of the nest was maintained at 33 C. The litters remained in isolation for 9 hours, after which they were returned to the home nest.

Housing

Each litter was weaned on PN28 and held in a breeding cage until all litters had been weaned, at which time they were moved out of the breeding room and into assigned housing. The enriched housing consisted of two wire cages 16 x 24 x 13 inches which were attached to each other by way of a tunnel. The cages were furnished with a ramp leading to food hoppers, a plastic tunnel, a metal nesting box filled with sawdust and three to five toys. Each cage housed 8 same sex animals, and something about the cage was changed daily; food or water location changed or toys removed, added or rearranged.

Animals in the standard condition were housed in pairs in 11 x 17 x 9 inch wire hanging cages, equipped only with a food hopper and a water bottle.

All animals were housed in the same room on a 12/12 hour light/dark cycle (lights on 2200) and fed ad lib on standard laboratory chow. The housing arrangement was maintained until the animals were 90 days of age. At this time 2 animals from each group were sacrificed for later anatomical verification and all remaining animals were placed in pairs in 11 x 17 x 9 inch plastic cages for the duration of the behavioral testing.

Behavioral Testing

T-Maze A wooden T-maze was constructed that consisted of three 24 inch open arms with recessed food wells at their ends. The maze was coated with a polyethelene finish that could be wiped clean between trials. The maze was located in a 10 by 9 foot room, and distinctive cues served to define specific locations within the room. Each animal was first given several adaptation trials. These trials were run until the animal ate all the Fruit Loops placed on the maze in five minutes or less on two consecutive trials. This was followed by 3 trials used to determine a side preference. Each animal was then trained to go to the side opposite its preference to find a food reward (a piece of Fruit Loop cereal). A correction procedure was used on the first 3 training trials: the animal was left on the maze until it found and ate the food. After this the animal was removed from the maze following an incorrect turn, except when it had been removed without food on 3 consecutive trials. If this happened the animal was allowed to remain until it found the food on the next trial. Each animal continued training until it reached a criterion score

of 8 correct responses in 10 trials. A tally was kept for each animal which indicated the number of correct responses over the total number of responses (e.g., the first trial was recorded 0/1 indicating 0 correct out of 1 trial, or 1/1; 1 correct out of 1 trial. In the event that an animal reached a point where it was impossible to achieve 9/10 (e.g. 4/7), counting started over on the next trial. The number of training trials each animal required to reach criterion was noted, as was the average latency for the animal to run the training trials. Once criterion was reached, a probe trial was conducted. In this trial the maze was rotated 180 degrees, and the animal was placed in the start arm and allowed to run to one food well. Scoring was as follows: "P" indicated a "place" strategy; that is the animal made an opposite turn from that used during training and visited the arm that was in the same physical place in the room where the animal had previously been reinforced. An "R" was recorded to indicate a response strategy in the event that the animal made the same turn (right or left) previously correct and thus searched in a different place in the room for the food. An "N" was recorded when an animal failed to move from the start arm or eat on the maze after three days of adaptation trials. Previous research has shown that handled/isolate animals more often use a response strategy, while nonhandled controls prefer a place strategy (Nadel and Willner, in press). This is consistent with the hippocampal decline seen in senescence (Barnes, Nadel and Honig, 1980).

Rotating Bar - Because the neonatal manipulations that were

used held the possibility of interference with the production and migration of granule cells, this task was used to assess the changes in the development of the cerebellum. Each animal was placed on a 24 inch long, 4 inch diameter bar that was rotated at speeds of 8 and 10 RPMs during training trials that lasted for 10 seconds. There were 10 training trials given on each of 2 training days. Following the training trials on the second day, two probe trials were given that lasted for 60 seconds each, the first was at a speed at 12 RPM, the second at 20 RPM. The latency of the animal to fall from the bar into a pool of water 18 inches below the bar was recorded.

Water Tank - Place Task A 6 ft. diameter fiberglass tank painted an off-white color was used for this task. The tank is fitted with a stable platform that offers the animal escape from constant swimming and can be moved to any of the four quadrants of the tank. Mounted at a height of 5 ft. above the tank is a video camera that projects an image to an HVS system, which in turn feeds data on-line to an Apple computer. Using these data the latency of the animal to escape to the platform, the distance traveled and the amount of time spent in various segments of the tank were recorded.

In the place version of the task, the platform remains in one location throughout the training phase (8 trials per day for 2 days), while the start location of the animal is varied in a quasi-random fashion. All possible combinations of the four start locations were generated on a computer and two of these combinations were randomly selected for use as start locations.

This assured that all animals started twice from each location, but the order was randomized. Efficient performance on this version of the task requires that the rat learn where the platform is located relative to extramaze cues, which as in the T-maze task were salient cues placed around the room. In adult animals the PLACE learning version of the maze requires a functioning hippocampal system (Morris, Garrud, Rawlins and O'Keefe, 1982).

Following 2 days of training, the animals received a "probe" trial, in which the platform was removed from the tank, and the rat was allowed to search for 60 seconds. This was done to measure the rat's spatial bias with the assumption that the rat would be biased toward the location where it expected to find the platform.

Water Tank - Cue Task The cued version of the water maze provides a salient cue above the platform. The cue and platform are moved on each trial in a quasi-random fashion determined in the same manner as the start locations in the place task. The animal can solve this version of the task by learning to approach the cue in the water maze, and does not need to learn about the location of the platform at all. Indeed efficient performance on the cued version of the water maze would almost seem to require that the rat learn to ignore the location of the platform within the tank. Lesions of the hippocampus have little, if any impact on the rat's ability to solve this version of the water maze task.

The training procedure for the cue task was the same as that for the place task. The animals were given 8 trials per day for 2 days. For the cue task the start location of the rat was the same

for all trials, but the location of the platform and the cue that marked it were changed on every trial. Again, a probe trial was given in which the cue remained, but the platform was removed from the tank.

RESULTS

Behavioral Testing

T-maze - The measures collected from the T-maze included the strategy used on the probe trial, the number of training trials the animal required to reach criterion, and the average latency on all training trials for each animal. Thirteen animals failed to adapt to the maze within 5 days and were not trained. Of the remaining 35 animals, 21 used a response strategy and 14 used a place strategy. There were no differences between groups on the strategy used (See Table 1).

The number of training trials each animal required to reach criterion was measured. Males needed more trials than females. Handled animals required more trials than nonhandled animals. Enriched and standard animals ran the same number of training trials (Table 2). A 3-factor (handling by housing by sex) ANOVA was computed. The only significant difference was sex, $F(1,27)=4.92, p<.05$.

The average latency for training trials was computed. The latency for males was longer than for females. Handled animals ran faster than nonhandled animals, and enriched animals ran faster than standard housed animals. A housing by sex interaction was found on the latency measure. While there was very little sex

difference in animals reared in enriched environments, standard housed animals showed a large sex difference (Table 3). A 3-factor ANOVA was computed. The sex difference was significant, $F(1,27)=4.49$, $p<.05$, as was the housing by sex interaction, $F(1,27)=5.43$, $p<.05$. Handling and housing were not significantly different.

Rotating Bar - All animals were first given 2 days of training on the bar at a rotation speed of 10 RPMs. Data collected for the rotating bar task were measures of the animal's latency to fall from the bar on 2 probe trials. On the first probe trial the bar was rotated at 12 RPM's and the animal was removed if it remained on the bar for 60 seconds. The second probe trial was conducted at a rotation speed of 20 RPM's and again was terminated at 60 seconds. Latency scores were averaged for each group and a 3-factor (handling by housing by sex) ANOVA was computed. Handled animals remained on the bar longer than nonhandled animals at both speeds. Enriched animals stayed on the bar slightly longer than standard housed animals at 12 and 20 RPM's. Females stayed on the bar longer than males (Table 4). The difference due to handling was significant at 20 RPM's, $F(1,36)=7.43$, $p<.05$, as was the sex difference at both speeds $F(1,36)=55.51$, $p<.05$. Housing did not significantly affect this measure. It was not surprising that females remained on the bar longer than males considering that the weight of the animal was highly correlated to latency to fall, $-.709$ at 12 RPM's and $-.689$ at 20 RPM's. There was a significant weight difference between males (535 grams) and females (293

grams), $F(1,42)=620.58$, $p<.05$. The weight difference between handled and nonhandled animals was not significant, $F(1,42)=2.54$, $p=.118$, although nonhandled animals tended to weigh more (439 gm) than handled animals (387 gm). Similarly enriched animals tended to outweigh standard housed animals (429.4 and 398.7) although this difference was not significant.

Water Tank - Place Task - Acquisition of place learning was measured by combining latencies into 2-trial blocks and looking at the resulting learning curve. These curves can be seen in figures 1 and 2. A 3-factor repeated measures ANOVA (group by sex by latencies) was performed. Females had lower latencies across trials than males. Whereas males tended to have fairly smooth curves, the female's latencies took several sharp drops. The group differences were not significant. The sex difference was significant, $F(1,280)=4.69$, $p<.05$.

The percentage of probe trial time that each animal spent searching in the quadrant where the platform had been located during training was analyzed. Handled animals spent 48.04% of the 60 second trial in the previously correct quadrant, while nonhandled animals spent 44.42% in that quadrant. Enriched animals spent 49% of the time in the correct quadrant, while standard housed animals spent 44.14% of the time there (Fig. 3). None of these factors were significant when analyzed with a 3-factor ANOVA.

The second measure analyzed for the place task was the carry-over from the first to the second day of training. This was done by computing an average for the last 2 trials on the first training

day and the first 2 trials on the second training day. Handled animals had a slightly higher latency on day 2 than day 1, while nonhandled animals had a slightly lower latency on the second day. Enriched animals had a longer latency on day 2 than day 1, while standard animals had a shorter latency on day 2. (Table 5). Males had similar latencies for days 1 and 2, while females slightly decreased their latency on day 2. A 4-factor (handling by housing by sex by trials) repeated measures ANOVA showed no significant differences, although housing by repeated measures approached significance, $F(1,44)=4.03$, $p=.051$.

Water tank - Cue Task - The same measures were obtained for the cued version of the water tank as for the place version. The first measure was the acquisition of cue learning (Figs. 4 and 5). Training trials were averaged in blocks of 2 for the 16 trials. Males had lower latencies than females across all training trials (9.7 for males and 12 seconds for females). Enrichment affected the handled and nonhandled animals differently. While latencies decreased for nonhandled animals that were enriched, latencies increased for handled animals that were enriched. Finally, while latencies dropped off quickly in early trials for the standard housed animals, later trials indicated that enriched animals learned faster. A 3-factor repeated measures ANOVA was computed (Handling by housing by sex by trials). The difference in latency for males and females was significant, $F(1,31)=5.97$ $p<.05$, as was the difference of sex by trials, $F(7,217)=2.46$, $p<.05$. The interaction between housing and handling was significant on both

the average latency difference and the difference across trials, $F(1,31)=7.85$, $p<.05$ and $F(7,17)=3.52$, $p<.05$. The difference across trials by housing was significant, $F(7,217)=2.71$, $p<.05$.

The percentage of time that an animal spent on the probe trial searching for the platform under the cue was measured. The cue was hung over one quadrant and the platform removed. Handled animals spent 50.5% of their time under the cue, while nonhandled animals spent 47.99% searching under the cue. Enriched animals swam in the cued quadrant 46.9% of the time, while standard housed animals spent 52.75% of their time in that quadrant. Males spent more time in the cued quadrant (55.94%) than females (43.0%). Handling and housing interacted on the percentage of time animals spent in the cued quadrant; enriching slightly increased the time in the correct quadrant for nonhandled animals (48.27% for enriched, 47.61% for standard), while handled animals spent more time in the cued quadrant when reared in standard environments (45.67% enriched, 57.89% standard). Figure 6 shows a graph of these results. A 3-factor ANOVA indicated significant differences of sex, $F(1,31)=16.58$, $p<.05$, and the handling by housing interaction, $F(1,31)=5.37$, $p<.05$.

The final cue measurement was of the differences among groups on the latency for the last 2 trials of the first day of training and the first 2 trials of the second training day. The nonhandled animals found the platform faster on day 2 than day 1, while handled animals increased their latency on the early trials of day 2. Enriched and standard animals both showed similar scores

across days 1 and 2, although enriched animals consistently had higher latencies than standard animals. Males improved their scores from the first to the second day, and females were slightly slower on day 2 (Table 6). Housing and handling interacted on the repeated measure. Handled animals had improved second day scores when they were reared in standard environments, but handled animals from enriched environments had higher latencies on day 2. A 4-factor (handling by housing by sex trials) repeated measures ANOVA was performed. The difference between enriched and standard animals was significant, $F(1,31)=6.33$, $p<.05$. The handling by housing by trials interaction was significant, $F(1,31)=6.11$, $p<.05$.

DISCUSSION

In general the hypothesis that the handling/isolation procedure used in this study would create deficits in tasks requiring hippocampal mediation was not supported. There do seem to be some trends in the data that suggest that the handled animals were somewhat impaired on learning compared to nonhandled animals. If the number of training trials that each animal required to reach criterion on the T-maze is taken as an index of errors committed, it can be seen that handled animals ran the maze faster (their latency scores were lower), but they committed more errors than nonhandled animals. This would seem to support the theory that handled animals are less fearful, or less emotional than nonhandled animals (Levine, 1959; Meyers and Fox, 1963). It also suggests that this is a task on which handled animals are impaired relative to nonhandled animals in learning ability. It would seem

impossible to separate the emotional from the learning components on this task. However, one possibility for the lack of expected findings on the strategy measure is the failure to properly adapt the animals to the test situation. As Harlow (1971) speculated it may be important to insure that all animals are equally adapted to the test situation before attempting to make any statements about learning differences. Therefore it seems that this task demonstrated emotional differences between handled and nonhandled controls, and hinted at learning differences between the groups, but for methodological reasons failed to pick up specifically hippocampal differences.

A provocative finding from the cue task suggests that the antithesis of the original hypothesis may be closer to the truth. Rather than ameliorating deficits due to handling, rearing in enriched environments appeared to exacerbate what were small differences in task performance for standard housed animals.

In summary this study found sex differences on all behavioral measures. Females ran faster and made fewer errors on the T-maze, stayed on the rotating bar longer than males (probably because they weighed less than males), and demonstrated more place than cue learning in the water tank, while the opposite was true for males. Enrichment appeared to increase performance on most tasks. Enriched animals ran faster and made about the same number of errors on the T-maze as standard animals. Enriched animals stayed on the rotating bar longer than standard animals. Enrichment also resulted in better performance on the place version of the water

maze. Evidence of cue learning was decreased for enriched animals however, on both the carry-over from day 1 to day 2 and the percentage of probe trial time spent under the cue, relative to standard housed animals.

The handling procedure showed the weakest and most inconsistent effects. Handled animals ran faster and made more errors on the T-maze, stayed on the rotating bar longer and showed increased performance on both measures of the place version of the water maze. On the cue task handled animals did better than nonhandled animals on the probe trial, but worse on the carry-over from day 1 to day 2. This again may suggest too much noise and too much variability within the experimental design.

Future research should aim at separating the effects of handling, isolation and enrichment, especially as they pertain to effects on learning ability. It would also seem wise to carefully evaluate the tasks used to measure learning, and to understand what the task is actually measuring as well as the emotional demands of a given task. Whereas the emotional differences that result from manipulation of the early environment are readily apparent, the learning differences are far more subtle and will be detected only through careful study.

A final important question for future research is the relationship between emotionality and learning. Although the Yerkes-Dodson law goes a long way in explaining how arousal levels (which are transitory) affect performance, it is unclear how permanent differences in neural mechanisms dealing with the stress

response system, alter the general learning abilities of an organism.

Table 1

Strategy used on Probe Trial in T-Maze

		<u>None</u>	<u>Strategy Place</u>	<u>Response</u>
Handled, Standard	Male	3	2	1
	Female	1	2	3
Handled, Enriched	Male	1	1	4
	Female	1	2	3
Nonhandled, Standard	Male	2	3	1
	Female	2	1	3
Nonhandled, Enriched	Male	1	2	3
	Female	2	1	3
Totals:		13	14	21

Table 2

Mean number of Training Trials on the T-Maze*

		<u>T-Maze Training Trials</u>		
		<u>Nonhandled</u>	<u>Handled</u>	<u>Totals:</u>
Enriched	Male	23.4 3.4	29.6 6.8	26.5
	Female	18 5.8	19 5.3	18.6
Standard	Male	21.5 9.2	27.7 6.6	24.1
	Female	22 8	21.6 3.5	21.8
Totals:		21.4	24.1	22.8

*The second number indicates standard deviation for each group.

Table 3

Mean Latency in Seconds for Training Trials on the T-Maze*

		<u>T-Maze Latencies</u>		<u>Totals:</u>
		<u>Nonhandled</u>	<u>Handled</u>	
Enriched	Male	33.94 6.8	66.96 51.3	50.45
	Female	68.47 36.7	37.54 16.8	51.29
Standard	Male	115.85 39.3	74.1 33.6	97.96
	Female	57.12 33.9	23.88 8.1	38.66
<u>Totals:</u>		66.79	48.01	57.13

*Standard deviations are noted below each latency.

Table 4

Mean Latency in Seconds for each Group to Fall from the Rotating Bar*

		<u>Latency to Fall</u>	
		<u>12 RPM</u>	<u>20RPM</u>
Enriched	Male	30	23.7
	Handled	16	8.4
	Female	57.7	60
		5.2	0
	Male	26.7	18.8
	Nonhandled	15.9	5.8
Standard	Female	57.7	52.8
		5.2	6.3
	Male	21	22.8
	Handled	8.7	16.1
	Female	35.8	31.8
		6.3	12.4
Standard	Male	11.2	3.7
		22.3	3.8
	Nonhandled	40	30.5
	Female	0	14.6

* Standard deviations are listed below latencies.

Table 5

Average Latencies from Day 1 to Day 2 on the Water Tank - Place Task*

		<u>Average Latency</u>	
		<u>Tr. 7-8</u>	<u>Tr. 9-10</u>
	Male	30.67	31.83
		18.7	22.5
	Handled		
	Female	22.5	27.33
		17.5	15.0
Enriched			
	Male	26.75	35.58
		10.1	17.5
	Nonhandled		
	Female	14.42	24.67
		6.4	10.7
	Male	34.92	30.75
		18.8	15.2
	Handled		
	Female	37.75	38.83
		14.4	10.4
Standard			
	Male	31.92	29.33
		10.6	11.9
	Nonhandled		
	Female	41.17	20.75
		15.7	15.5

*Average latencies in seconds for the last 2 trials of the first training day (Tr 7-8) and the first 2 trials of the second day (Tr 9-10).

Table 6

Average Latencies from Day 1 to Day 2 on the Water Tank - Cue Task*

		<u>Average Latency</u>	
		<u>Tr. 7-8</u>	<u>Tr. 9-10</u>
	Male	9.5	10.5
		4.1	5.1
Handled			
	Female	10.33	19.0
		4.7	7.7
Enriched			
	Male	12.8	8.6
		10.1	3.8
Nonhandled			
	Female	15.66	12.0
		7.6	7.5
	Male	9.25	6.75
		2.3	2.1
Handled			
	Female	7.75	6.5
		2.6	2.5
Standard			
	Male	7.0	7.5
		3.0	3.8
Nonhandled			
	Female	10.5	10.5
		2.4	1.7

*Latencies in seconds for the last 2 trials of the first training day (Tr 7-8) and the first 2 trials of the second day (Tr 9-10), for the cued version of the water maze.

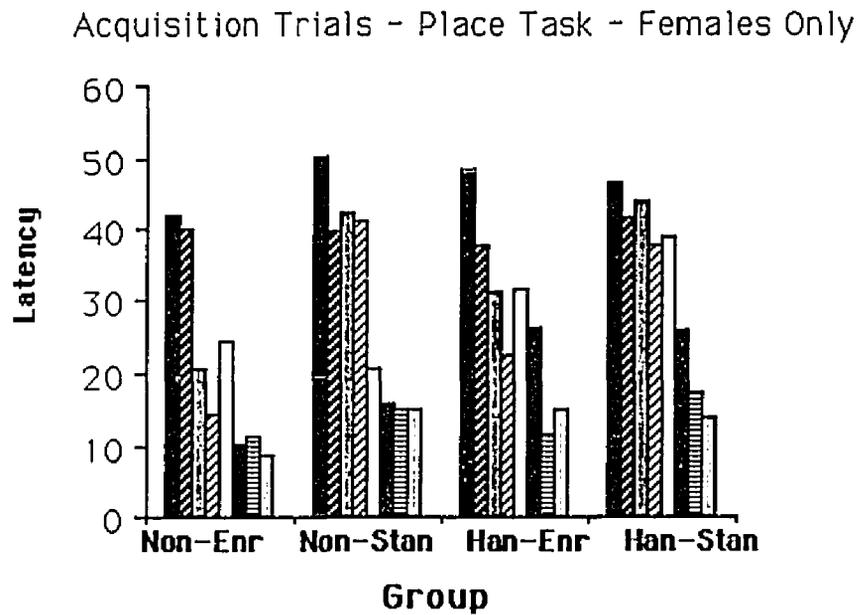


Figure 1 Average latency in seconds for females on the acquisition trials for the place version of water maze.

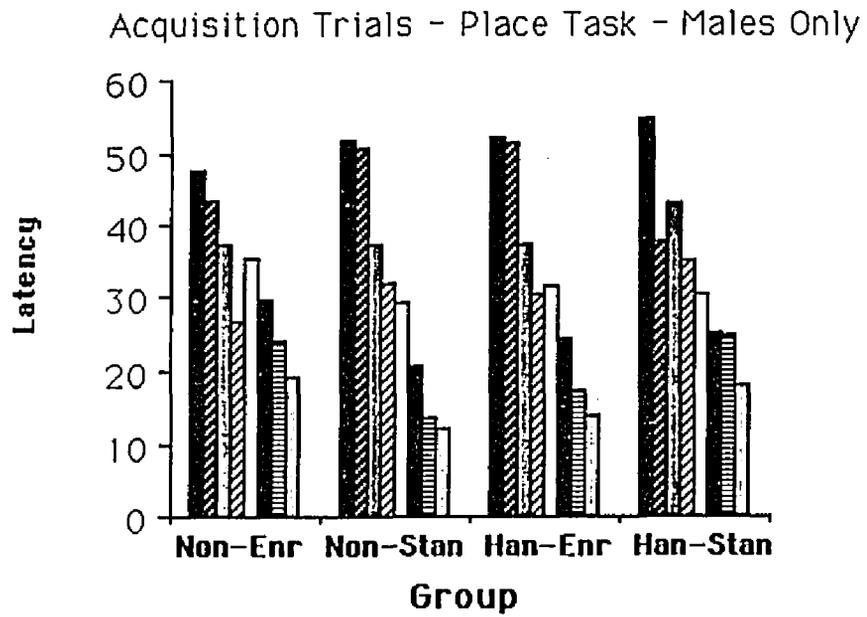


Figure 2 Average latency for males on acquisition trials in the place version of the water maze.

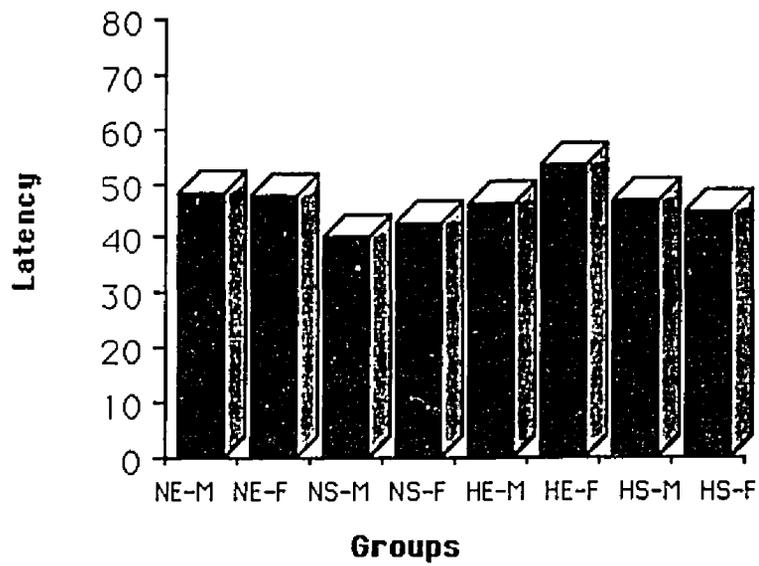
Percentage of Time in Expected Quadrant - Place Task

Figure 3 Percentage of time spent in the previously correct quadrant on the probe trial of the place task.

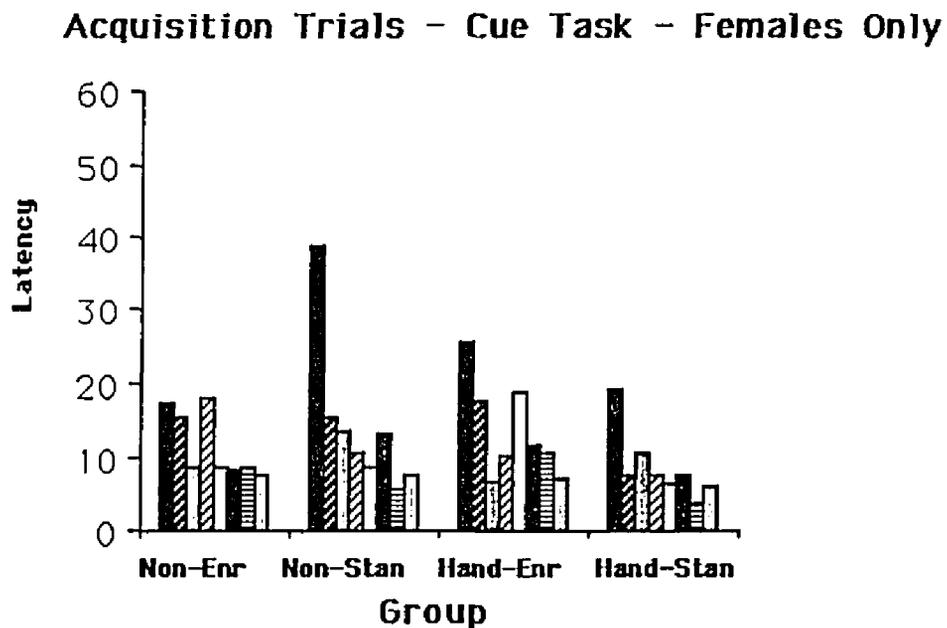


Figure 4 Average latency in seconds for females on acquisition trials for the cued version of of the water maze.

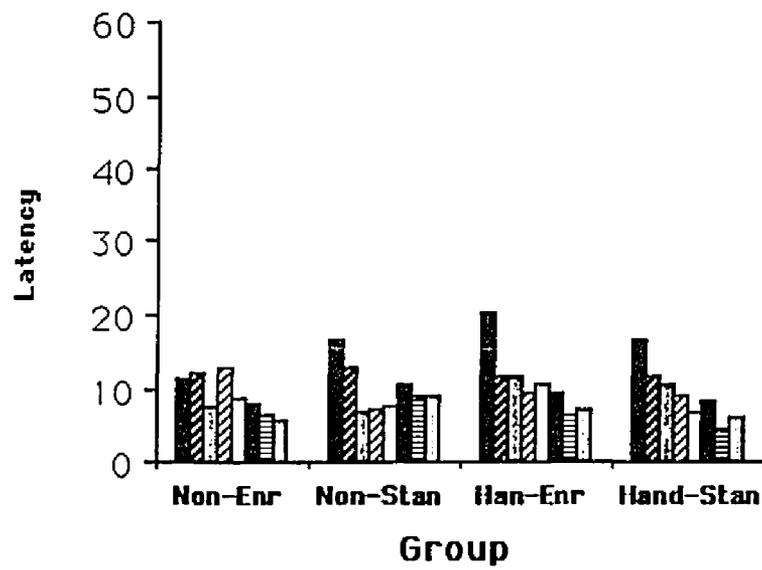
Acquisition Trials - Cue Task - Males Only

Figure 5 Average latency in seconds for males on acquisition trials for the cued version of the water maze.

Percentage of Time in Correct Quadrant - Cue Task

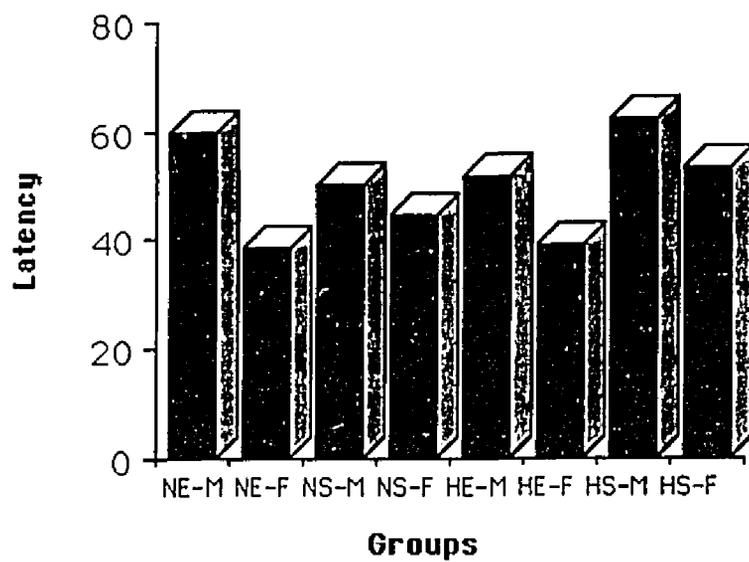


Figure 6 Percentage of time spent in the cued quadrant on the probe trial of the cue task.

*FULL REVIEW*ULACC Control No. 85-0013

CERTIFICATION OF ANIMAL RESEARCH PROTOCOL REVIEW

University of Arizona - University Laboratory Animal Care Committee

Project Title: Long-Term Effects of Early Handling/Isolation on Hippocampal
Cognitive FunctionFunding Agency: March of Dimes Birth Defects FoundationPrincipal Investigator: Lynn NadelDepartmental Address: Psychology/Cognitive SciencePsychology DepartmentUniversity of Arizona, Main CampusPhone Number 621-5497

I. VETERINARY REVIEW

COMMENTS:

The experiments proposed will be conducted in a manner so as to not put undue stress on the animals. No painful procedures will be performed on the animals. Euthanasia methods are appropriate.

Approved: _____

Disapproved: _____

Revision Requested: _____

Signature, Veterinarian10/31/85
Date

II. UNIVERSITY LABORATORY ANIMAL CARE COMMITTEE REVIEW

COMMENTS:

This grant was unanimously approved by the ULACC as submitted at a convened full review.

Approved: ✓

Disapproved: _____

Revision Requested: _____

Signature, Chairman12/6/85
Date

7/1/85

REFERENCES

- Altman, J. and Das, G.D. (1965) Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *Journal of Comparative Neurology*, Vol 124, 319-336.
- Altman, J., Das, G.D. and Anderson, W.J. (1968) Effects of infantile handling on morphological development of the rat brain: an exploratory study. *Developmental Psychobiology*, Vol 1, 10-20.
- Angevine, J. (1965) Time of neuron origin in the hippocampal region, an autoradiographic study in the mouse. *Experimental Neurology Supplement*, Vol 13, 2, 1-70.
- Barnes, C.A. (1988) Spatial learning and memory processes: the search for their neurobiological mechanisms in the rat. *Trends in Neuroscience*, Vol 11 (4), 163-169.
- Barnes, C.A., Nadel, L. and Honig, W.K. (1980) Spatial memory deficit in senescent rats. *Canadian Journal of Psychology*, 34, 29-39.
- Bayer, S.A. (1980) Development of the hippocampal region in the rat. I. Neurogenesis examined with ³H-thymidine autoradiography. *Journal of Comparative Neurology*, Vol 190, 87-114.
- Becker, J.T., Walker, J.A. and Olton, D.S. (1980) Neuroanatomical bases of spatial memory. *Brain Research*, Vol 200, 307-320.
- Bennett, E.L., Rosenzweig, M.R. (1979) Behavioral and biochemical methods to study brain responses to environment and experience. In: Rosenzweig, M.R. and Bennett, E.L. (Eds.) *Neural mechanisms of learning and memory*. The MIT Press.

- Bohn, M. (1985) Glucocorticoid induced teratologies of the nervous system. In: Yanai, J. (Ed.) Neurobehavioral Teratology. Elsevier Science Publishers, BV.
- Bronson, G. (1974) The postnatal growth of visual capacity. *Child Development*, Vol 45, 873-890.
- Chevalier, J.A. and Levine, S. (1955) The effects of shock and handling in infancy on adult avoidance learning. *The American Psychologist*, Vol 10, 432.
- Crain, B., Cotman, C., Taylor, D. and Lynch, G. (1973) A quantitative electron microscopic study of synaptogenesis in the dentate gyrus of the rat. *Brain Research*, Vol 63, 195-204.
- Daly, M. (1973) Early stimulation of rodents: a critical review of present interpretations. *British Journal of Psychology*, Vol 64 (3), 435-460.
- Denenberg, V.H. and Morton, J.R.C. (1962) Effects of environmental complexity and social groupings upon modification of emotional behavior. *Journal of Comparative and Physiological Psychology*, Vol 55 (2), 242-246.
- Denenberg, V.H., Rosenberg, K.M., Haltmeyer, G.C. and Whinby, A.E. (1969) Programming life histories: effects of stress in ontogeny upon emotional reactivity. *Merrill-Palmer Quarterly*, Vol 10, 109-116.
- Diamond, M.C. (1988) Enriching heredity: The impact of the environment on the anatomy of the brain. The Free Press, A Division of Macmillan, Inc. New York.

- DuPreez, P.D. (1964) The persistence of some effects of handling in infancy on the behavior of the adult rat. *Quarterly Journal of Experimental Psychology*, Vol 16, 147-155.
- Eckenhoff, M.F. and Rakic, P. (1988) Nature and fate of proliferative cells in hippocampal dentate gyrus during the life span of the Rhesus monkey. *The Journal of Neuroscience*, Vol 8 (8), 2729-2747.
- Engellenner, W.J., Goodlett, C.R., Burright, R.G. and Donovan, P.J. (1982) Environmental enrichment and restriction: Effects on reactivity, exploration and maze learning in mice with septal lesions. *Physiology and Behavior*, Vol 29, 885-893.
- Harlow, H.F. and Zimmermann (1959) Affectional responses in the infant monkey. *Science*, Vol 130 (3373), 421-432.
- Harlow, H.F., Harlow, M.K., Schiltz, K.A. and Mohr, D.J. (1971) The effect of early adverse and enriched environments on the learning ability of Rhesus monkeys. In: Jarrard, L.E. *Cognitive Processes on Nonhuman Primates*. Academic Press. New York.
- Hickey, T.L. and Peduzzi, J.D. (1987) Structure and development of the visual system. In: (Ed.) *Handbook of infant perception*, Volume 1. Academic Press, Inc.
- Hinde and Spencer-Booth (1971) Effects of brief separation from mother on Rhesus monkeys. *Science*, Vol 173 (3992), 111-118.

- Kirby, R.J., Stein, D.G., Kimble, R.J. and Kimble, D.P. (1967)
The effects of hippocampal lesions and duration of sensory
input on spontaneous alternation. *Journal of Comparative
Physiological Psychology*, Vol 64, 342-345.
- Kretschmann, H.J., Kammradt, G., Krauthausen, I., Sauer, B. and
Wingert, F. (1986) Growth of the hippocampal formation in
man. *Biblioteca Anatomica*, Vol 28, 27-52.
- Leaton, R.N. (1965) Exploratory behavior in rats with
hippocampal lesions. *Journal of Physiological Psychology*, Vol
5, 331-334.
- Levine, S. (1959) The effects of differential infantile
stimulation on emotionality at weaning. *Canadian Journal of
Psychology*, Vol 13 (4), 243-247.
- Levine S., Lewis, G.W. (1959) Critical period for effects of
infantile experience on maturation of stress response. *Science*,
Vol 129, 42-43.
- Levina, S., Lewis, G.W. (1959) The relative importance of
experimenter contact in an effect produced by
extra-stimulation in infancy. *Journal of Comparative
Physiological Psychology*, Vol 52, 368-369.
- Levine, S. (1962) The effects of infantile experience on adult
behavior. In: Bachrach, A.J. (Ed.), *Experimental Foundations of
Clinical Psychology*. New York: Basic Books.

- Meaney, M.J.; Aitken, D.H., Bodnoff, S.R., Iny, L.J.
and Sapolsky, R.M. (1985) The effects of postnatal handling on the development of the glucocorticoid receptor systems and stress recovery in the rat. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* Vol 9, 731-734.
- Meaney, M.J., Aitken, D.H., Bodnoff, S.R., Iny, L.J.,
Tatarewicz, J.E., Sapolsky, R.M. (1985) Early postnatal handling alters glucocorticoid receptor concentrations in selected brain regions. *Behavioral Neuroscience*, Vol 99 (1), 765-770.
- Melzack, R. and Thompson, W.R. (1956) Effects of early experience on social behavior. *Canadian Journal of Psychology*, Vol 10, 82-90.
- Meyers, R.D. and Fox, J. (1963) Differences in maze performance of groups vs. isolation reared rats. *Psychological Reports*, Vol 12, 199-202.
- Micco, D.J. and McEwen, B.S. (1980) Glucocorticoids, the hippocampus, and behavior: interactive relation between task activation and steroid hormone binding specificity. *Journal of Comparative and Physiological Psychology*, Vol 94, 624-633.
- Morris, R.G.M. (1984) Development of a water-maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods*, Vol 11, 47-60.

- Morris, R.G.M., Garrud, P., Rawlins, J.N.P. and O'Keefe, J.
(1982) Place navigation impaired in rats with hippocampal lesions. *Nature*, Vol 297, 681-683.
- Nadel, L. and Willner, J. (In Press) Some implications of postnatal maturation of the hippocampus.
- O'Keefe, J. and Nadel, L. (1978) *The hippocampus as a cognitive map*. The Clarendon Press, Oxford.
- Munck, A., Guyre, P.M. and Holbrook, N.J. (1984) Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocrine Reviews*, Vol 5, 25-44.
- Osbourne, B., Sivakumaran, T., Black, A.H. (1979) Effects of fornix lesions on adrenocortical responses to changes in environmental stimulation. *Behavioral and Neural Biology*, Vol 25, 227-241.
- Rakic, P. (1985) Limits of neurogenesis in primates. *Science*, Vol 227, 1054-1055.
- Rakic, P. and Nowanowski, R.S. (1981) The time of origin of neurons in the hippocampal region of the Rhesus monkey. *Journal of Comparative Neurology*, Vol 196, 99-128.
- Rosenfeld, P., Sutanto, W., Levine, S. and DeKloet, E.R. (1988) Ontogeny of Type I and Type II corticosteroid receptors in the rat hippocampus. *Developmental Brain Research*, Vol 42, 113-118.
- Rosenfeld, P., Van Eekelen, J.A.M., Levine, S. and De Kloet, E.R. (1988) Ontogeny of the Type 2 glucocorticoid receptor in discrete rat brain regions: an immunocytochemical study. *Developmental Brain Research*, Vol 42, 119-127.

- Sapolsky and Meaney, M.J. (1986) Maturation of the adrenocortical stress response: Neuroendocrine control mechanisms and the stress hyporesponsive period. *Brain Research Reviews*, Vol 11, 65-76.
- Sarrieau, A., Sharma, S. and Meaney, M.J. (1988) Postnatal development and environmental regulation of hippocampal glucocorticoid and mineralocorticoid receptors. *Developmental Brain Research*, Vol 43, 158-162.
- Sarrieau, A., Vial, M., Philibert, D. and Rostene, W. (1984) In vitro autoradiographic localization of (3H)corticosterone binding sites in rat hippocampus. *European Journal of Pharmacology*, Vol 98, 151-152.
- Schlessinger, A.R., Cowan, W.M. and Gottlieb, D.I. (1973) An autoradiographic study of the time of origin and the pattern of granule cell migration in the dentate gyrus of the rat. *Journal of Comparative Neurology*, Vol 159, 149-176.
- Smith, A.M. (1967) Infantile stimulation and the Yerkes-Dodson law. *Canadian Journal of Psychology*, Vol 21, 285-293.
- Spence, J.T. and Maher, B.A. (1962) Handling and noxious stimulation of the albino rat: I. Effects on subsequent emotionality. *Journal of Comparative and Physiological Psychology*, Vol 55 (2), 247-251.
- Stanton, M.E., Wallstrom, J. and Levine, S. (1987) Maternal contact inhibits pituitary-adrenal stress responses in preweanling rats. *Developmental Psychobiology*, Vol 20 (2), 131-145.

- Stanton, M.E. and Levine, S. (1989) Maternal modulation of infant glucocorticoid stress responses: role of age and maternal deprivation. *Psychobiology*, Vol 16 (3), 223-228.
- Sturrock, R.R., Smart, J.L. and Tricklebank, M.D. (1983) A quantitative neurohistological study of the long term effects in the rat brain of stimulation in infancy. *Journal of Anatomy*, Vol 136 (1), 129-144.
- Sutherland, R.J. and Dyck, R.H. (1984) Place navigation by rats in a swimming pool. *Canadian Journal of Psychology*, Vol 38 (2), 322-347.
- Turner, B.B. (1978) Ontogeny of glucocorticoid binding in rodent brains. *American Zoologist*, Vol 18, 461-475.
- Wiesel, T.N. and Hubel, D.H. (1965) Comparison of the effects of unilateral and bilateral eye closure on cortical unit responses in kittens. *Journal of Neurophysiology*, Vol 28, 1029-1040.
- Wilson, M.M., Greer, S.E., Greer, M.A. and Roberts, L. (1980) Hippocampal inhibition of pituitary-adrenocortical function in female rats. *Brain Research*, Vol 197, 433-441.
- Wilson, M., Warren, J.M. and Abbott, L. (1965) Infantile stimulation, activity, and learning by cats. *Child Development*, Vol 36, 843-853.
- Wilson, D.A., Willner, J., Kurz, E.M. and Nadel, L. (1986) Early experience alters brain plasticity. *Behavioural Brain Research*, Vol 21, 223-227.

- Wong, R. (1966) Infantile handling and performance in the T-maze. *Psychonomic Science*, Vol 5 (5), 203-204.
- Wong, R. and Jamison, J.L. (1968) Infantile handling and the facilitation of discrimination and reversal learning. *Quarterly Journal of Experimental Psychology*, Vol 20, 197-199.