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Rearing condition affects the development of allocentric responding and hippocampal N-methyl-D-aspartate receptors in rats

Hyatt, Laura Elizabeth, Ph.D.

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U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

REARING CONDITION AFFECTS THE DEVELOPMENT OF ALLOCENTRIC RESPONDING AND HIPPOCAMPAL N-METHYL-D-ASPARTATE RECEPTORS IN RATS

by

Laura Hyatt

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Rats were reared in one of two conditions; RESTRICTED and OPEN. Rats reared in the RESTRICTED condition were reared without access to stable patterns of visual landmarks and rats reared in the OPEN condition had access to such landmarks. The rats reared in the RESTRICTED condition did not exhibit allocentric spatial behavior when tested at age 52 days using a 4-trial habituation paradigm while rats in the OPEN condition did respond allocentrically. Further, the rats reared in the RESTRICTED condition failed to respond allocentrically during a second day of testing when the test required them to use landmarks on the floor rather than on the walls of the arena. However, the RESTRICTED rats did respond egocentrically when tested a third time, indicating that their failure to respond allocentrically during the first two tests was probably not due to a general inability to notice changes in object location. Hippocampi of rats reared in the RESTRICTED and OPEN conditions were examined using [^{3}H] TCP kinetic binding analysis to determine if the rats differed in number or response of NMDA receptors. They did not. Additionally, hippocampal slices from rats in both conditions were electrically stimulated and population EPSP's measured to determine any difference in NMDA receptor response to electrical stimulation. The NMDA receptors in RESTRICTED

animals contributed much less to the population EPSP than the NMDA receptors of OPEN animals. In conclusion, restricted access to stable patterns of visual landmarks delays or prevents the development of allocentric behavior in rats and results in hippocampal NMDA receptors that respond differently to electrical stimulation. It is hoped that this research will lead to the development of a mammalian model for studying the mechanisms by which experience alters behavioral and neural development.

ACKNOWLEDGEMENTS

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CHAPTER I

INTRODUCTION

Since the 1950s, researchers studying spatial behavior have focused on adult animals, particularly rats (O'Keefe and Nadel, 1978; Olton, 1979; Tomlinson, 1989). Much more recently, researchers have concentrated on describing the series of changes that constitutes the development of spatial orientation, focusing mainly on human infants (Pick and Lockman, 1981; Acredelo, 1978; Bremner and Bryant, 1977). Over the past six years have researchers emphasized the study of how animals develop adult orientation behavior, rather than simply chronicling when in development certain behaviors appear (Gustafson, 1984; Tomlinson, 1989; Bai and Bertenthal, 1990).

The research on adult spatial behavior served to thoroughly characterize the phenomenon and has made it clear that the class of behaviors identified as spatial behavior is important for animals' survival because it is crucial for locating food, conspecifics, nest sites, and hiding places (Kamil and Balda, 1985; Jamon and Bovet, 1987; Schenk, 1987). The study of spatial behavior took on additional importance for psychologists when it came to be viewed by some as a

form of memory that could be studied both in animals and humans (O'Keefe and Nadel, 1978). Finally, researchers began to turn their attention to the development of spatial behavior because it offered a useful avenue for studying the development of memory in infants (Acredelo, 1978; Bremner and Bryant, 1977; Pick and Lockman, 1981) and animals (Tomlinson, 1989; Castro and Rudy, 1987). Research on the development of spatial behavior has served to identify some of the experiences important for normal development and the sequence of behaviors that are ordinarily observed during normal development (Bai and Bertenthal, 1990; Tomlinson, 1989; Acredelo, 1978; Gustafson, 1984; Bremner and Bryant, 1977; Castro and Rudy, 1987). However, there is little research (Castro and Rudy, 1987) designed to investigate the mechanisms by which experience alters the development of spatial behavior.

The argument for pursuing research on developmental mechanisms goes beyond the issue of how complete an explanation needs to be. Understanding more about the mechanisms by which sensory stimulation alters the development of behavior could contribute greatly to the formulation of general principles of development. In addition, an increased understanding of developmental mechanisms may provide new therapeutic techniques in cases where humans or animals are not developing normally.

The lack of research on mechanisms of the development of

spatial orientation is probably due to a variety of influences. Certainly not all researchers are interested in the development of spatial behavior or the mechanisms of that development. However, it is also true that there has traditionally been a widespread assumption that spatial behaviors are innate (Zuckerman and Rock, 1957; O'Keefe and Nadel, 1978, pp. 52-55). As frequently happens, once a characteristic is labeled innate, further research into developmental mechanisms is deemed unnecessary (Kuo, 1920; Lehrman, 1953; Oyama, 1985; Johnston, 1987). This occurs primarily because under the dichotomous theoretical framework (in which traits are either innately or environmentally determined) once a feature is said to be genetically determined, it is assumed that the only important question left can be answered by identifying the genes that code for the trait and perhaps determining whether the inheritance of the trait follows Mendelian patterns (Oyama, 1985; Johnston, 1987; Upchurch and Wehner, 1989). Developmental issues may be abandoned once the work is turned over to molecular or population geneticists. However, more and more over the past thirty years researchers have found this approach to development inappropriate (Kuo, 1920; Lehrman, 1953; Oyama, 1985; Gottlieb, 1983; Johnston, 1987, Schneirla, 1966). Further, an increasingly sophisticated understanding of how genes function has supported the argument that development is not best captured by the "either genes or environment"

analysis (Johnston & Hyatt, in prep).

As a more complex picture of development emerges, some researchers avoid making the assumption that any characteristic (or aspect of a characteristic) can be either genetically or environmentally determined. One result of this non-dichotomous approach to development is that researchers are less likely to ask whether a characteristic is influenced by the genes and are more likely to ask by what mechanisms animals change during development. As previously mentioned, the development of spatial behavior is one research domain in which studies of developmental mechanisms have been lacking. Most researchers interested in spatial behavior have concentrated on adult behavior (Kamil and Balda, 1985; Jamon and Bovet, 1987; Menzel, 1973; 1978, Morris, 1981; O'Keefe and Nadel, 1978; 1971; Olton, 1976) and those interested in development have focused on when adult-like behavior appears rather than how it develops (Acredelo, 1978; Pick and Lockman, 1981; Bremner and Bryant, 1977).

The literature on adult animals suggests that there are two kinds of spatial behavior: egocentric and allocentric (Pick and Lockman, 1981; Tomlinson, 1989). Egocentric spatial behavior is based on the animals' body location alone, without reference to an external framework; allocentric behavior is based on an external framework (Acredelo, 1978). The distinction between egocentric and allocentric responding is similar to the one made earlier by Tolman (1948) between

response learning and place learning. An animal may initially learn to turn left in a +-maze to locate a goal. Animals exhibiting response learning turn left when placed in the maze, regardless of the starting position. In contrast to response learning, Tolman described place learning as behavior based on an external framework of objects or a "map". In the case of place learning, an animal does not simply learn to turn left in the +-maze to locate a goal, but instead learns to locate the goal based on the relationship between the goal and the rest of the objects in the framework. More recently, researchers interested in spatial behavior have adapted Tolman's distinction between place learning and response learning to identify allocentric spatial behavior and egocentric spatial behavior, respectively (Bremner and Bryant, 1977; Acredelo, 1978; Tomlinson, 1989).

The primary consequence of this distinction for developmental research has been the identification of the ages at which infants and young animals behave egocentrically or allocentrically; little attention has been paid to the question of how these abilities develop. Only very recently have researchers begun to investigate what factors play a role in development from egocentric to allocentric spatial behavior, as opposed to simply assuming that spatial behavior patterns will emerge according to some pre-determined maturational schedule (Zuckerman and Rock, 1957; O'Keefe and Nadel, 1978; Upchurch and Wehner, 1989). Conditions found to

influence the development of localization abilities include mother's nuturing style in hamsters (Tomlinson, 1989), delayed crawling in infants (Bai and Bertenthal, 1990), early malnutrition in rats (Castro and Rudy, 1986), and environmental complexity in rats (Juraska, et al., 1983; Einon, 1980).

In addition to this work on spatial behavior in young animals, data on the neural basis of spatial behavior in adult animals have been accumulating rapidly (McNaughton et al, 1986; Meck, et al., 1988, 1989; Morris, et al., 1986; Morris, 1989; Petit, 1988; Castro and Rudy, 1987; Olton, et al., 1977; Lynch et al., 1983; Maier, et al., 1990). This study was designed to take advantage of this growing understanding in order to investigate the mechanisms by which early experience affects the development of spatial orientation. Work on the development of the central nervous system indicates that sensory stimulation alters its structural and functional properties, perhaps even its DNA activity (Hyden and Egyhazi, 1962; Grouse, et al., 1980; Rose, 1990) in important ways. Research on the neural basis of spatial behavior has implicated the hippocampus (Sutherland and Rudy, 1989), and particularly N-methyl-D-aspartate (NMDA) receptors in that structure (Morris, 1981, 1989; Morris, et al., 1986), in the production of adult patterns of spatial behavior. This study was designed to investigate <u>how</u> sensory stimulation interacts with other factors to alter behavioral and neural development.

Therefore, this project was designed to (1) determine the role of certain kinds of early experience in the development of adult patterns of spatial behavior in rats and (2) determine whether the structure and function of the hippocampus may be influenced by that early experience.

Brief History of the Concepts of Spatial Behavior

Very early experiments on the learning of maze habits (Small, 1901; Watson, 1907) were interpreted to mean that rats learned to navigate a maze by using a stimulus-response chain based on proprioceptive input. In these studies, the spatial component of the rats' behavior was not of special interest; the researchers were studying general behavioral principles. However, Watson and Lashley (1915) did use the terms proximal and distal localization to describe bird navigation, implying that at least in birds, the problem of spatial orientation in particular was of interest. According to their definition, distal orientation involves finding an object that is not directly detectable by vision, hearing, or sense of smell; that is, no spatially concurrent cues are available to guide an animal to the object. Thus, the only way the animal can find the object is by using a chain of responses that ultimately leads it to the goal. In contrast, proximal localization involves orienting toward an object which is visible or otherwise directly detectable by the senses. For instance, an animal might follow an odor gradient to an unseen

source of food. So, according to Watson and Lashley (1915) locating objects involved either stimulus-response chains or movement toward objects already detectable by some sensory modality.

However, some researchers began to focus on the spatial component of maze learning as spatial behavior because their research indicated that rats could not learn difficult mazes on the basis of stimulus-response chains or concurrent cues (Dashiell and Helms, 1925; Honzik, 1936). The factor common to these (and other) studies which indicated that rats were not simply learning response chains or following odor gradients was the flexibility rats exhibited if allowed to enter the maze from a new direction. If the rats were simply learning a series of left-right turns (a stimulus-response chain), entry into an old maze from a new direction should result in the rats' repeating the same left-right sequence they learned originally; they would not, therefore, successfully complete the maze. Instead, the rats were often able to complete the maze regardless of starting position. In order to understand what the rats were learning that allowed them to exhibit such flexibility, Dashiell (1930) and Honzick (1936) conducted extensive studies on the sensory basis of maze navigation in rats. They concluded that vision plays a dominant role in navigating mazes open to the environment (mazes the animals could see out of) and that olfactory, tactile, and auditory stimulation were less

important when vision was available to the animal. In fact, when Watson (1907) rotated open mazes 180° relative to the surrounding environment, his rats had difficulty running a maze they had previously learned. This finding was later interpreted to mean that rats used extramaze cues during problem-solving (O'Keefe and Nadel, 1978, p. 50).

The rats' use of these extramaze environmental cues helped explain the flexibility they exhibited. Tolman (1932; 1948) proposed that rats (and animals in general) form cognitive maps. By this he meant that they base their behavior on frameworks of environmental relationships that allow them to respond flexibly in the maze. Tolman called maze behavior based on environmental relationships place learning, and contrasted it with response learning in which an animal locates goals in its environment simply by learning to turn left or right regardless of its position in relation to the goal. Tolman's use of the term cognitive map and his use of the distinction between place- and response- learning (Tolman, 1932; 1948) helped to focus attention more narrowly on the problem of spatial orientation behavior (Woodworth, 1938) and provided a way to think about the way in which animals use the spatial configuration of environmental cues to orient.

Subsequently, many of the distinctions that have been made among different kinds of spatial behavior have built on this basic distinction between place and response learning. For instance, Harrison and Nissen (1941) distinguished two

kinds of spatial localization: absolute localization was defined in terms of the observer's body position (like response learning) and relative localization was defined in terms of a stable framework outside the observer's body (like place learning). Piaget (1954, 1971) used the concept of egocentricity to describe the behavior of young children who do not appear to take the perspective of others into account. Thus, according to the Piagetian model, egocentric children solve spatial problems based on their own body location, not on an objective framework of cues, again a distinction similar to that between response learning and place learning. In one last example of Tolman's influence, Potegal (1969) refers to egocentric spatial localization as observer-based localization; that is, localization dependent on the observer's position.

Bremner (1978) used the original place-versus-response distinction to describe the basis for errors young infants make in spatial tasks. Subsequently, Bremner and Bryant (1978) replaced that distinction with one between allocentric and egocentric responding. According to Bremner and Bryant (1978), egocentric behavior involves locating objects in relation to an animal's body position whereas allocentric behavior involves locating objects in relation to stable frameworks of cues. The egocentric/allocentric distinction is the same as the distinction between visual discrimination learning (egocentric) and place navigation (allocentric) that

workers such as Morris (1989) use in reference to rats' performance in the Morris water maze.

Spatial Behavior Patterns in Adult Animals

O'Keefe and Nadel (1978) cite several advantages that allocentric localization may provide over egocentric behavior. One of the most fundamental may be that responses based on an objective frame of reference provide flexibility. No matter how the animal itself is oriented, allocentric responding permits it to locate objects of interest quickly and accurately. The location of objects based on stable frames of reference is important for a wide variety of animals and has been a topic of investigation in a host of different species including hamsters (Poucet et al., 1986), gerbils (Thinus-Blanc and Ingle, 1985), rats (Schenk 1987; Hymovitch, 1950; Olton, 1978), Olton and Samuelson, 1976; Olton and Schlosberg, 1978), mice, (Jamon and Bovet, 1987), primates (Menzel, 1973; Menzel, 1978) and birds (Balda, 1980; Kamil and Balda, 1985).

Much research designed to demonstrate spatial behavior in adult animals is accomplished by training animals to locate objects under one set of circumstances and then observing where the animals look under a new set of circumstances. For example, in one seed-caching study (Kamil and Balda, 1985), birds were allowed to hide seeds next to certain landmarks and then while the birds were not looking, all the landmarks (but not the seeds) were shifted a specific distance in a specific

direction. The birds then searched (unsuccessfully) for the seeds near the landmarks, rather than where the seeds were actually hidden, indicating that the association between the hiding place and the landmarks was guiding their food recovery.

Apparently, adult rats do not need to see or otherwise sense an object in order to locate it if they have been allowed to learn about its location in relation to a stable framework of objects they can see. Morris (1981) investigated this ability in rats by devising a pool of opaque water equipped with a moveable escape platform. Rats were trained to swim to the escape platform from a fixed position in the maze. If the maze was uncovered during training and testing, the rats were quite good at locating a hidden platform when placed in the maze from different start points. However, if access to the stable framework of cues was blocked with a black curtain during testing, the rats had great difficulty locating the hidden platform. Analysis of where the animals spent the most time searching for the platform indicated that they were responding egocentrically, because they tended to turn in the direction (right or left) that worked during training (Morris, 1981; Morris, 1989). This work indicates that adult animals may respond egocentrically or allocentrically as the situation demands.

The preceding studies required the animals to make an oriented response to their environment, but spatial

localization behavior has also been investigated by noting changes in exploratory behavior when object configuration has been altered in some way. Montgomery (1953) reported that animals will exhibit more exploratory behavior in an unfamiliar stimulus situation than in a familiar one. From studies which use a change in the amount of exploratory behavior exhibited under old and new circumstances as an index of what animals attend to, object configuration has emerged as an important stimulus for exploratory behavior. Several authors have found that animals will increase exploratory behaviors in a given situation if the spatial layout of objects changes (Wilz and Bolton, 1971; Thinus-Blanc and Ingle, 1985). For example, Thinus-Blanc and Ingle (1985) allowed gerbils to explore an arena containing an object, then measured changes in their exploratory behavior after the animals entered the arena from a new direction. They recorded an increase in exploration of the object. Poucet et al., (1986) allowed hamsters to habituate to a particular spatial arrangement, then noted an increase in exploratory behavior after the objects were rearranged. Dishabituation of exploration has been used as an indicator that animals detect changes in spatial relationships among objects. The cues animals use to orient in the arena need not be within the arena itself. Animals may also use landmarks outside the arena such as lights, wall panels, or wires on the ceiling (Poucet, et al., 1986). This habituation paradigm is

particularly useful for developmental work because the task is so simple that the animals do not have to be trained prior to testing and the tests themselves can be very short in duration. These features reduce the possibility that testing itself will interfere with the developing system under investigation.

One common understanding about adult spatial behavior that has come out of this work is that frequently (although not always) animals appear to use the spatial relationships among objects to orient in the world. Evidence from work with infants and young animals however, indicates that the ability to behave allocentrically is not present at birth. Young animals may instead employ the sometimes less efficient egocentric response strategy in locating objects and exploring the environment. The aim of this project was to investigate the effect of early experience on the development of allocentric responding in rats.

Development of Spatial Behavior Patterns

Work on egocentric/allocentric responding has determined that humans and non-human animals begin to exhibit allocentric behavior sometime after birth. For instance, Acredelo (1978) reports that human infants respond egocentrically between 6 and 11 months old and shift to allocentric behavior around 16 months of age. That is, young infants trained to expect an event to occur to their left or their right continue to

respond based egocentrically even when they are moved to a new location 180° away from the training position. However, at around 16 months of age, infants are able to orient toward the event of interest when their location in the room is changed. In similar tests, Bremner (1978) and Pick and Lockman (1981) report that human infants first respond egocentrically and that allocentric behavior does not begin until later in development. In addition, recent work with young hamster pups 14-18 days old, using an habituation paradigm, indicates that allocentric behavior does not develop until about 15-16 days of age, although 14-day-old pups do exhibit egocentric localization behavior (Johnston et al., in prep).

These studies suggest that egocentric behavior generally develops before allocentric behavior. However, knowing the sequence of development is not enough to understand how certain behaviors develop. It is also important to understand what mechanisms may contribute to the development of allocentric behavior. For example, rats reared in an enriched environment (toys, other rats present, large space, transparent or translucent cages) learn to negotiate mazes faster and more accurately than rats reared in impoverished environments (no toys, isolated, smaller space, translucent or opaque cages) (Forgays and Forgays, 1952; Juraska et. al., 1983). However, the superiority of the enriched rats over the impoverished rats disappeared if the maze they learned was

rotated between trials (Brown, 1968; Forgays and Forgays, 1952; Hymovitch, 1952). Apparently, enriched rats were using the visual cues above and around the maze and this may account for their superiority over the impoverished rats. When the maze was rotated, external landmarks were unreliable, forcing both the enriched and impoverished rats to use the same egocentric strategy and so perform equally well (Juraska, et al., 1983). This finding indicates that something about the impoverished condition is affecting the development of allocentric behavior. However, because researchers have used a range of different circumstances to produce the impoverished and enriched conditions, it is difficult to ascertain exactly what it is about the environment that is influencing development. Bai and Bertenthal (1990) report that if infants are prevented from crawling at the usual time because of orthopedic devices, they do not respond allocentrically when normal same-aged infants do. The hamster pups of dams reared on a liquid diet and that are themselves reared on a liquid diet do not respond allocentrically when same-aged control pups do (Tomlinson, 1990). Tomlinson asserts that the inability to respond allocentrically may be tied to the fact that the pups of liquid-diet mothers apparently spend less time exploring away from the nest than pups reared by normal-diet mothers. He suggests that early exploratory experience may be important for normal development of allocentric behavior. Further, malnutrition during early life slows the development

of allocentric behavior in rat pups, but malnourished pups still exhibit egocentric behavior in a Morris water maze (Castro and Rudy, 1986). In this case, the authors hypothesize that the behavioral difference is due to a difference in hippocampal development.

Perhaps one common thread running through all of this work on the development of spatial behavior is that in all cases the animals or infants with behavioral deficits may have had reduced or greatly modified self-produced exploratory experience. The infants in orthopedic devices were restrained from crawling at the normal age (Bai and Bertenthal, 1989), the hamster pups of liquid-reared dams spent less time exploring on their own (Tomlinson, 1989), rats reared in impoverished environments (Forgays and Forgays, 1952; Hymovitch, 1952; Brown, 1968) had a diminished stimulus array to explore compared to enriched controls, and the malnourished rats (Castro and Rudy, 1986) may have spent less time exploring their surrounds than properly nourished controls. Together, these studies suggest that early in life some aspects of sensory stimulation during exploration are important for the normal development of adult spatial behavior patterns.

Identifying significant early experiences alone is not sufficient for understanding how animals develop allocentric behavior. In order to attain a more thorough understanding of spatial behavior development it is also important to

investigate the neural correlates of spatial behavior so that ultimately, we can begin to understand the mechanisms by which sensory stimulation contributes to developmental changes. If animals reared in different conditions develop different spatial behavior, then presumably they will exhibit different neural development as well. Correlating behavioral development with neural development will advance our understanding of how different experiences act to produce differences in behavior, because it is the neural systems that process the experiences in question. Fortunately, the neural correlates of spatial behavior in adult animals have been under investigation for quite some time. This work on the neurophysiology of adult spatial behavior provides an excellent springboard for research into how differences in early experience can result in differences in the development of allocentric behavior.

The Role of the Hippocampus in Spatial Behavior

The hippocampus has been implicated in behavioral tasks involving spatial configuration (O'Keefe and Nadel, 1978; Green, 1964; Douglas, 1964). For example, Rabe and Haddad (1969b) demonstrated that rats with hippocampal lesions have difficulty learning the location of water in a plus maze, even when provided with numerous extramaze cues. The same rats had no difficulty learning the location of the water before the hippocampal lesions or after a sham operation. In addition, Plunkett et al.(1973) trained normal and hippocampal

lesioned rats on two tasks: an egocentric task (go right regardless of starting point) and an allocentric task (find the place regardless of starting point). Rats with hippocampal lesions learned the egocentric task as rapidly as the controls but had more difficulty learning the allocentric task. Olton and Isaacson (1968) performed an experiment suggesting that damage to the hippocampus may alter aspects of spatial behavior. Rats with hippocampal lesions were impaired relative to normal rats in the acquisition and retention of a one-way active avoidance task. Olton and Isaacson suggest that the hippocampectomized rats are worse in the one-way avoidance procedure because they have difficulty associating the shock with the cues indicating spatial location of the shock.

The radial-arm maze is frequently used to study spatial behavior. Normal rats can successfully learn a radial-arm maze, as evidenced by the fact that a rat will rarely visit previously visited arms, while rats with hippocampal lesions frequently exhibit random choice patterns (Olton et al., 1977). Similarly, in the Morris water maze, hippocampus-damaged rats can learn to find a platform if they are allowed to see it while they are learning its location and if its location does not change from training to testing, a condition that permits egocentric behavior to be successful. However, they cannot learn to find a hidden platform if they must respond allocentrically, using extramaze cues to navigate

(Morris et al., 1986). Similar findings have been observed in primates, which show a deficit in place learning after hippocampectomy but not before (Douglas and Pribram, 1966.

However, Olton et al. (1979) have argued that the role of the hippocampus is specific not to tasks involving spatial configuration but to those involving working memory as opposed to reference memory. This conclusion is based on the finding that hippocampal damage did not alter performance when rats used extramaze cues and intramaze cues, although it did differentiate between performance when rats were required to use working rather than reference memory. In this study, Olton et al. assumed that egocentric behavior necessarily depends on the use of cues inside the test arena and that allocentric behavior necessarily depends on cues outside the arena. However, it has been argued (Bures, 1979) that there is no functional distinction between extramaze and intramaze cues, as long as the intramaze cues are not directly associated with the goals of the maze in space, and that Olton et al. did not use a test that actually required the animals to use two kinds of spatial orientation. Both intramaze and extramaze cues are simply patterns that animals may use to navigate. So, while it may be that the hippocampus is important for more than just spatial memory, for the purposes of understanding the role of the hippocampus in spatial orientation tasks, it appears useful to distinguish between tasks that do require the use of object configuration

(allocentric responding) and tasks that do not (egocentric responding) (Sutherland and Rudy, 1989).

More recent literature involving selective impairment of the hippocampus supports the idea that the hippocampus plays an important role in allocentric responding. For example, Deyo and Conner (1989) report that leupeptin (a protease inhibitor) administered to the rat hippocampus impairs acquisition of the eight-arm radial maze. Further, Alessandri et al. (1989) administered ketamine, a drug that blocks NMDA receptors in the rat hippocampus, and tested the rats' ability to find a platform in the Morris water maze. If animals had to rely on the configuration of objects overhead to find the platform, their performance was impaired but if they simply had to remember to go left or to go right relative to their starting position, they were able to accomplish the task. Morris (1989) reports similar results after the administration of the drug AP5 (a potent competitive NMDA antagonist). Rats administered AP5 did not use the configuration of objects outside the water maze to locate a hidden platform, although rats administered only artificial cerebrospinal fluid can do this quite easily. Morris speculates that his result may be due to the fact that AP5 blocks an outcome of synaptic transmission called long-term potentiation (LTP) in the area of the hippocampus thought to be important for these kinds of spatial tasks.

Hippocampal NMDA Receptors, LTP, and Spatial Behavior

Many synapses among central neurons are excitatory and many are understood to use excitatory amino acids (L-glutamate and L-aspartate) as neurotransmitters. Although there are five known receptors for excitatory amino acids, the N-methyl-D-aspartate receptor (NMDA receptor) is the most thoroughly understood (Monaghan and Cotman, 1989). These receptors are activated by either aspartate or glutamate and are largely found in brain cortical regions associated with higher-order processing (Cotman et al., 1989; Cotman et al., 1987).

NMDA receptors participate in a phenomenon known as long-term potentiation (LTP) (Brown et al., 1988; Cotman et al., 1989). LTP is a long-lasting increase in the efficiency of a post-synaptic response following a brief period of high-frequency (tetanic; 100-400Hz) stimulation at the afferent fibers (Bliss and Lømo, 1973) (Figure 1). Because LTP can last up to several days it is thought to be one of the neural mechanisms underlying memory (Cotman, et al., 1989; Morris, 1989). Characteristics of LTP other than its stability, such as synaptic specificity and its dependence on neural firing patterns, make LTP a promising mechanism for memory (Cotman et al., 1989). LTP has been observed in several different brain regions, but has been most thoroughly studied in the hippocampus (Teyler and Discenna, 1987).

The mechanism for LTP is not well understood (Cotman et

al. 1989). Evidence supporting both pre- and post-synaptic mechanisms is present in the literature. Some lines of evidence suggest that the influx of calcium ions through post-synaptic NMDA channels sets off a series of biochemical changes in the post-synaptic cell which lead to LTP (Baudry, et al., 1981; Lynch, et al., 1983) (Fig. 2). The increase in calcium ions in the post-synaptic cell may activate calcium-dependent kinases which, in turn, may increase the responsiveness of the post-synaptic cell (Lynch and Baudry, 1984). Additionally, the NMDA receptor has a binding site for the amino acid glycine which acts to potentiate the response of the NMDA receptor by increasing the frequency with which the calcium channel opens (Cotman, et al., 1987). Also, evidence exists that NMDA receptors act in concert with other glutamate receptors, particularly quisquilate receptors (Cotman, et al., 1987).

Increased neurotransmitter release after LTP initiation suggests that pre-synaptic mechanisms may also be involved in LTP (Dolphin, Errington, and Bliss, 1982). It has been suggested that even though the site of LTP induction appears to be post-synaptic, a post-synaptically activated signal may be relayed back to the pre-synaptic cell to produce the potentiation (Bliss and Lømo, 1983). Finally, much evidence suggests that LTP results in permanent changes in synapse structure, such as the formation of more, larger, and perforated synapses (Petit, 1988). If this is the case, it

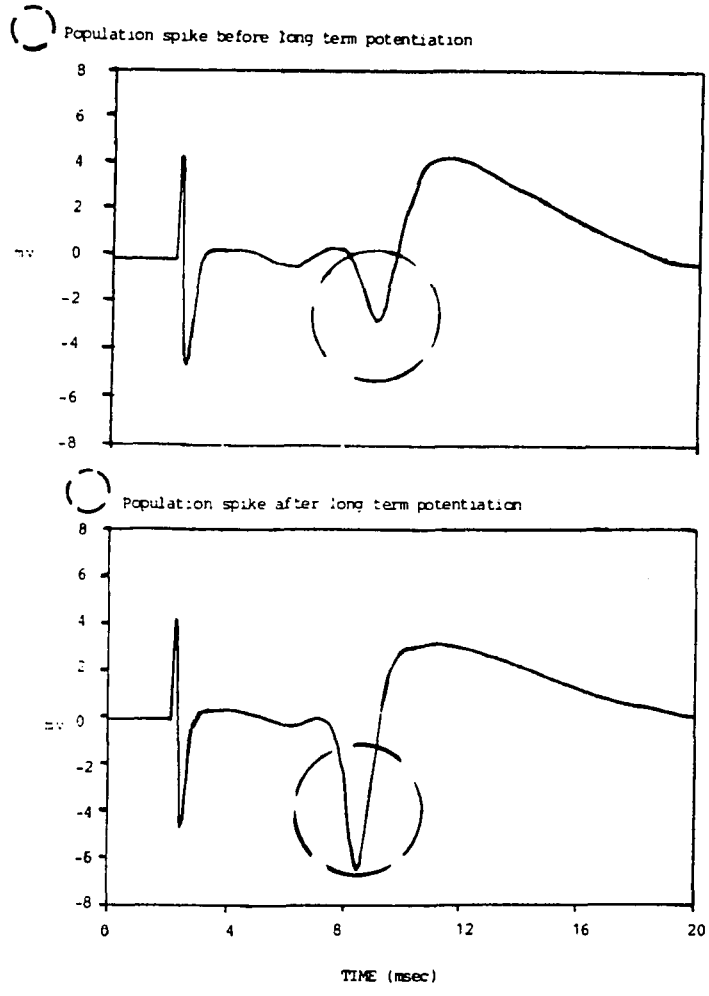

Figure 1. A schematic illustrating long-term potentiation. A change in the electrical activity of neurons can be seen by measuring the population spike (action potentials of many cells recorded extra-cellularly). (A) Response of the neurons to stimulation before potentiation as measured by the slope of the circled population spike. (B) Response of the neurons after potentiation. This change in the population spike is brought about by high-frequency stimulation (100-400 Hz) but can be evoked with low-frequency stimulation for weeks after the initial tetanus.

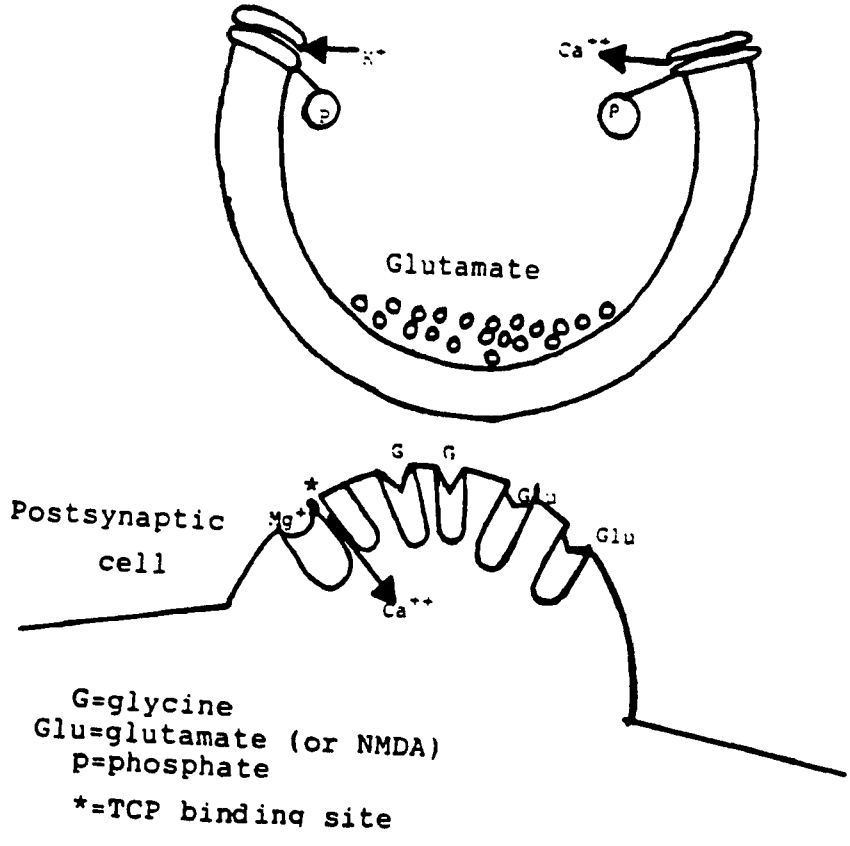

Figure 2. Schematic of N-methyl-D-aspartate receptor on post-synaptic cell. The presence of magnesium blocks the calcium channel but magnesium block is removed with depolarization oif the cell. Calcium must pass through the channel into the cell for long-term potentiation to occur.

is likely that both pre- and post-synaptic changes are involved in LTP and memory (Cotman, et al., 1989; Petit, 1988; Chang and Greenough, 1984).

As previously mentioned, current data strongly suggest a connection between spatial memory and LTP in the hippocampus. For instance, rats can quite easily learn to perform a hippocampus-dependent task. However, if LTP is induced with electrical stimulation, rats are unable to acquire a new spatial task, although they can still perform previously learned mazes. According to McNaughton et al. (1986) the electrical stimulation saturates the ability of the neurons to exhibit LTP. He suggests that any specific modification in the circuitry of the neural pathways required for learning the new maze cannot be accomplished because of the LTP they induced. As already noted the administration of the potent NMDA antagonist, AP5, interferes with the ability of the rats in his 1990 study to learn a Morris water maze based on configurational cues, although AP5-treated rats can still learn a simple right-left discrimination. Morris (1990) also reported that AP5 actually interfered with LTP induction in the hippocampus. To summarize, hippocampal NMDA receptors have been shown to be important for the performance of spatial behavior tasks.

NMDA Receptors and Developmental Plasticity in the Hippocampus

Because of the relationship among NMDA receptors, LTP, and memory it seems likely that these receptors play an important role in neurobehavioral plasticity. Evidence from studies of physiological responses in the immature rat hippocampus indicate that early post-natal experience could play a crucial role in hippocampal development and the behaviors associated with hippocampal functioning. For example, beginning on post-natal day 5, LTP can be elicited, and its expression peaks around day 15. After day 15, the ability to exhibit LTP declines rapidly until it reaches adult levels (Harris and Teyler, 1984). Further, during post-natal days 5-15 there is an increase in the number of hippocampal NMDA receptors (Tremblay, et al., 1989). From post-natal day 5 to day 9 rat hippocampal neurons are maximally responsive to the application of NMDA. Finally, NMDA receptors are more sensitive (more easily activated) in immature than in mature hippocampal neurons (Ben-Ari, et al., 1989). Together with what is understood about the structural changes that accompany LTP (i.e., changes in numbers, width, and perforation of synapses), this evidence suggests that early post-natal experience may be crucial for hippocampal and behavioral development. At this point, no one has combined behavioral and physiological approaches to investigate the mechanisms of the development of allocentric behavior. The purpose of this study was to investigate the effects of early experience on

the development of both allocentric behavior and NMDA receptors in the hippocampus.

Purpose of the Study

This project had two main pruposes: First to refine the work suggesting that early rearing experience affects the development of allocentric responding by rearing rats from birth in precisely defined conditions so that the primary difference in the two conditions was access to visual stimulation outside the rearing cage; second, to determine if this difference in rearing condition affected either the number of NMDA receptors in the hippocampus or the ability of the excitatory neurotransmitter aspartate to open the calcium ion channels. In addition, I wanted to investigate the possibility that glycine, an amino acid which enhances the response of NMDA receptors to stimulation, does not function to open the calcium channel on the NMDA receptors of the RESTRICTED rats. To confirm that NMDA receptors are involved in the test for allocentric responding, I also administered an NMDA antagonist (AP5) to a group of control rats (see below) and tested for a deficit in allocentric responding in the presence of the antagonist.

CHAPTER II

METHODS AND MATERIALS

I. **Subjects, colony maintenance and breeding procedures**

All subjects for the experiments described below were laboratory-born offspring of Lewis rats (Lewis isogenic strain; Lew/CrlBr) obtained from Charles River Laboratories. The animals were maintained in an indoor colony room in the Department of Psychology at UNC-Greensboro. Because the rats are nocturnal, the colony was maintained on a reversed day/night cycle (12L:12D), with the lights off at 10 am. The animals were housed in clear plastic cages (22cm x 42.5cm x 19cm) with wire tops and were provided with commercial corn cob bedding material. All animals were provided with food (Purina Rodent Chow) and water ad libitum.

Males and females were mated as necessary to provide litters for the rearing experiments described below. When pregnancy was confirmed, males were removed to separate cages. There were never more than 2 adult rats per cage unless a male was breeding with two females (these rats are polygynous). Food and water were checked daily and cages were cleaned weekly except that cages of dams nursing pups were not changed until the pups were weaned. Litters were culled (via halothane inhalation in a closed chamber) to 8 rat pups per dam when the pups were 1-2 days old. All rat pups were weaned

on day 21-22 (when they weighed 35-40 grams).

II. Behavioral Testing

A. General Experimental Apparatus and Procedures

1. Rearing Conditions

Litters in the OPEN condition were born and reared in clear plastic cages with wire tops (35.5cm x 50.8cm x 20.32cm). On postnatal day 21 the 8 pups in an OPEN litter were weaned into clear plastic cages identical to those they were born in, 4 animals to a cage, until testing on day 51-52. The cage tops for the OPEN condition allowed the animals to see the colony room ceiling. In addition, food and water were delivered from atop the cage so that the animals had to look up to eat or drink.

Litters assigned to the RESTRICTED condition were born in opaque white cages fitted with fine-mesh white cloth tops (35cm x 55cm x 37.5cm). The white mesh allowed air and diffuse light in, but prevented the animals from seeing out of the cage. On day 21 the 8 pups in a RESTRICTED litter were weaned into opaque white cages identical to those they were born in, 4 pups per cage, until testing on day 51-52. Water was delivered via a sipper tube projecting into the cage about 3 cm from the cage floor. To administer food the tops of the cages were lifted just enough to allow food pellets to be dropped to the floor. Therefore, with the exception of the sipper tube, the walls and ceiling of the RESTRICTED group

were a uniform white and the animals' attention was not directed upward by food or water administration for any longer than a few seconds every three days.

Animals in both conditions had access to small colored objects from weaning to testing. These objects were made of light-weight plastic which the rats could easily manipulate and move around the cage. As such, the objects were rarely in the same place for very long. Pilot data indicated that animals reared without access to objects exhibit greater variability in responding during testing, perhaps due to the novelty of encountering objects. All animals received food and water ad lib.

2. **Apparatus**

I carried out behavioral tests by placing the rats in a rectangular box 91cm x 61cm x 30cm that was painted a uniform flat black. This arena was open on top and there were numerous objects on the walls and ceiling of the test room that the animals could see from the arena. In addition, two 4 x 6 index cards, one with slanted black-and-white stripes and one with black-and-white checks on it, were taped on two walls of the arena to serve as additional, visually prominent cues. Two white objects (hand soap dispensers, coffee mugs, or flower vases, 8-12 cm tall, with either vertical or horizontal black stripes or large black dots) were placed 5 cm apart within a centrally located 7.5 x 15 cm^2 approach area

painted in white on the arena floor (Fig.3). Rats are more likely to approach patterns of high contrast (Karmel, 1969) and so such patterns were used wherever possible. Rats that were subjected to more than one test in the arena (see below) were exposed to different sets of objects in the arena on each test.

Testing was conducted under red light (25 watts) during the first five hours of the dark part of the light:dark cycle. I used two hand-held stopwatches to monitor trial length and record the amount of time the animals spent in the approach area.

3. **Procedure**

a. Allocentric Responding Using Visual Cues on the Arena Wall

The test for allocentric responding was a four-trial habituation paradigm. I used three litters of 8 animals (n=24) in each condition (OPEN and RESTRICTED) for a total of 48 rats. In trials 1-3 an animal was carried from its home cage in a covered box and placed in the arena at a designated entry point (Fig 3) with two visually distinct objects located within the approach area. In each trial the animal was left in the arena for 5 minutes and a stopwatch used to record the amount of time the animal spent with at least its snout in the approach area. (An animal facing out of the approach area with its snout outside of the approach area line was not

counted as in the approach area.) After 5 minutes, the animal was placed in the covered box for 3 minutes until the next trial. In trial 4, the location of the objects in the approach area was reversed and the animal entered the arena from a point 180^{o} away from the entry point in trials 1- 3 (Fig.3). As in the first three trials, I measured the amount of time the animal spent within the approach area. Allocentric responding was indicated by dishabituation on trial 4. Continued habituation on trial 4 indicated a lack of allocentric responding.

b. Allocentric Responding to Floor Cues

Failure to respond allocentrically in the previous test did not necessarily indicate that the rats would not respond allocentrically under any circumstances. It was possible that RESTRICTED animals would respond allocentrically if the cues were on the floor of the arena rather than on the ceiling and walls of the test room and arena. Recall that the RESTRICTED rearing condition included a water spout at eye level and food, bedding, and objects on the cage floor. To test for the possibility that RESTRICTED animals would respond allocentrically to floor cues, I used the previously described procedure to re-test the RESTRICTED animals for allocentric responding to visual cues on the floor instead of on the arena walls or on the ceiling of the test room. During all four trials, a conspicuous pattern of diagonal white stripes (4cm

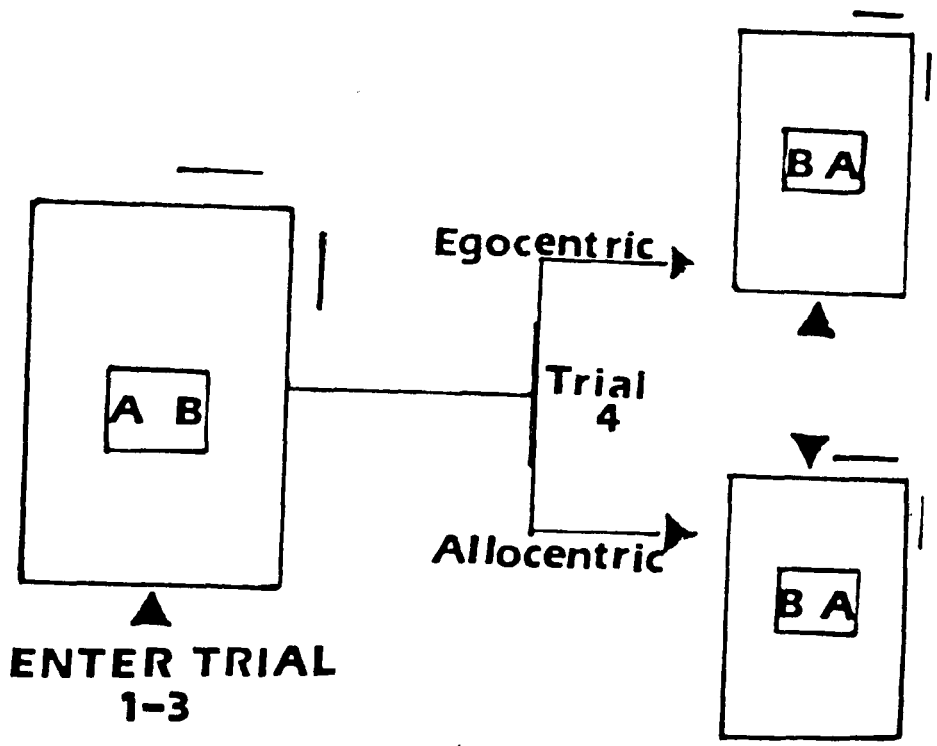

Figure 3. Schematic of arena during allocentric and egocentric testing. The heavy black arrows indicate the entry point in each trial.

x 4cm) was present on the arena floor to provide a source of configurational information. Data were scored and interpreted as in the first experiment.

c. Egocentric Responding

Animals in the RESTRICTED group were tested a third time to confirm that they would respond egocentrically to a spatial change in the arena. I used the same four-trial habituation paradigm as described for the first two experiments. In this test, however, the entry point on trial 4 was not changed, although object position was reversed (Fig.3). Dishabituation of exploration on trial 4 indicated that the animals responded egocentrically to the change in the position of the objects.

d. Effects of NMDA Antagonist AP5 on Allocentric Responding in OPEN Rats

To confirm that NMDA receptors play a role in the habituation paradigm the potent NMDA receptor antagonist AP5 was administered to the hippocampi of OPEN reared animals in order to test its effect on allocentric responding. If NMDA receptors are important for allocentric responding as I have defined it for this project, then the presence of AP5 should interfere with the allocentric responding in OPEN rats.

Six OPEN reared animals from three different litters were fitted with two small (21G1 1/2 Yale hypodermic needles) stainless steel guide tubes which penetrated the right and left hippocampi. This was accomplished by administering a

general anesthetic to the animals (Nembutal, 60 mg/kg; i.p.), and, using a stereotaxic holder to immobilize the head, drilling two small holes into the exposed skull overlying the locations of the hippocampi (-3.4 Bregma, 2.0 mm medial). The guide tubes were lowered through the skull 2 mm from the surface of the brain to a position just dorsal to the hippocampus (Fig. 4). The area around each guide tube was sealed with dental cement. A plug of stainless steel surgical wire was inserted into the tube while drug was not being administered to prevent fluid leakage. The animals were allowed to recover in their home cage for 72 hours before being tested. Behavioral testing proceeded as described previously for "Testing for Allocentric Responding to Ceiling Cues". However, prior to each trial a .001 mg/ml concentration of AP5 in 1 μl of artificial cerebrospinal fluid was administered to each side of the hippocampus with a Hamilton syringe. I waited 2-3 minutes following drug administration before behavioral testing began to ensure adequate drug absorption. In order to control for the effects of surgery and the drug administration procedure another six OPEN animals from three different litters were implanted with guide tubes and administered vehicle solution alone before behavioral testing.

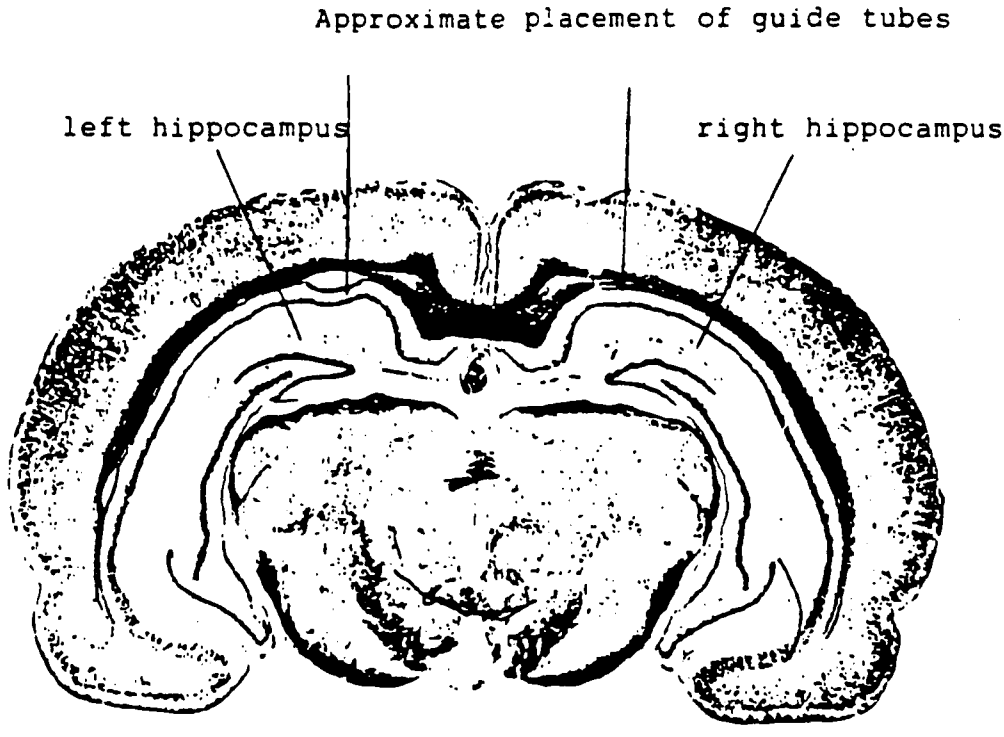

Figure 4. Diagram of cross-section of hippocampus showing placement of guide tubes for injection of AP5 and ACSF.

III. **Physiological Tests**

A. TCP Kinetic Binding Analysis

If the RESTRICTED rearing condition had any physiological effect, it may have affected any of several different aspects of NMDA receptor structure and functioning. It is particularly difficult to make predictions about the nature of the difference between the two groups because the mechanism for LTP at NMDA receptors is not well understood and because the structure of the NMDA receptor is complex and not completely elucidated (Cotman, et al. 1989). Based on what is currently understood about the structure and function of the NMDA receptor (see previous discussion pp. 23-27), I tested for two possible differences in the receptors between the OPEN and RESTRICTED animals: a difference in number of NMDA receptors in the hippocampus and a difference in whether or not NMDA and glycine opened the calcium channel of the NMDA receptor.

Due to the technical nature of the procedures involved, these tests required collaboration with Dr. Douglas Bonhaus and Dr. James NcNamara in the Department of Neurology at Duke University. For details not provided in the ensuing description, see Bonhaus and McNamara (1988); Bonhaus et al.(1989), and Yeh et al.(1990).

1. General Tissue Preparation

a. **Dissection**

In the interest of exercising control over the amount of

hippocampal tissue removed from each rat, Dr. Ken-ichi Ito dissected out the hippocampus of 8 RESTRICTED animals and 8 OPEN animals when they were 51-52 days old, while I assisted. The animals were killed via decapitation and their right and left hippocampi removed by blunt dissection in artificial cerebrospinal fluid (aCSF). The tissue was immediately frozen in liquid nitrogen and transferred in labeled containers to a -70^{o} C freezer to await transfer to Duke University for analysis.

b. **Membrane Preparation**

Each pair of hippocampi was homogenized in a polytron (setting number 6, for 20 secs) in 10 ml cold 50 mM Tris acetate buffer (pH 7.7) containing 1.0 mM EDTA in order to expose the synaptic membranes containing the binding sites for maximum binding during the tests. Following homogenization, the tissue was centrifuged at 10,000 g for 20 min at 4^{0} C. The tissue was washed with eight additional cycles of homogenization, resuspension in fresh buffer, and centrifugation [NOTE: washes 5-9 were performed with 5.0 mM Tris acetate buffer without EDTA (pH 7.2)]. Membranes were frozen in a methanol bath. Before the 5th centrifugation, the membranes were incubated for 15 mins at 37^{o} C. The membranes were stored frozen (overnight) after the third centrifugation until the day of the binding experiment.

1. Differences in Receptor Number

Following the techniques developed by Bonhaus and McNamara (1988; 1989) kinetic binding experiments were used to determine differences in receptor number. These techniques used a radioactively labeled non-competitive NMDA receptor antagonist called N-(1- thienyl cyclohexyl) piperidine (TCP) that binds to a site on the calcium channel of the NMDA receptor (Fig. 2). To determine differences in NMDA receptor number, the prepared membranes of 8 OPEN animals and 8 RESTRICTED animals were incubated with a fixed concentration of NMDA and glycine under equilibrium conditions. The amount of TCP bound over time was measured with a scintillator counter which measured the amount of radioactivity emitted from each sample. The amount of radioactivity emitted from each sample was in direct proportion to the amount of TCP bound which in turn was proportional to the number of NMDA receptors in the sample. Using the curve-fitting program LIGAND the value labeled B_{max} (maximum binding) was calculated for each pair of hippocampi. Since the number of receptors available for binding determines how much TCP is taken up by the membranes, B_{max} for the two groups of animals allows a comparison of the number of NMDA receptors in the two groups of membranes.

2. Differences in Receptor Response to NMDA and Glycine

A similar technique was performed to assess any

difference in the effectiveness of NMDA or glycine at opening the Ca^{++} ion channels between the two groups of animals. In this case membranes from 4 OPEN animals and 4 RESTRICTED animals were prepared as described previously but then incubated with progressively greater concentrations of NMDA and glycine and the concentration of bound TCP was measured every two minutes for a period of ten minutes. When NMDA and glycine are present and bound to the receptor, Ca^{++} ion channels are opened and TCP binding to that channel increases. If the effectiveness of NMDA and glycine were low, the receptors would not be activated and TCP binding would be reduced. Again, using the computer program LIGAND the mean EC_{50}(effective concentration) for NMDA and glycine was determined. The higher the EC_{50},the lower the effectiveness of the NMDA and glycine at opening the ion channels of the NMDA receptors. This technique allowed a comparison of the responsivity of calcium channels of NMDA receptors to NMDA and glycine without confounding it with NMDA receptor number.

B. Extracellular Recording to Determine Differences in NMDA Receptor Response to Stimulation

This part of the study was designed to investigate the possibility that the response of the NMDA receptor to low frequency stimulation in the hippocampal slice preparation is different for RESTRICTED and OPEN animals. Due to the technical nature of this work, these tests required

collaboration with Dr. Ken-Ichi Ito. For this work, extracellular recording techniques were used to compare the contribution of NMDA receptors to the slope of the excitatory post synaptic potential (EPSP) in OPEN and RESTRICTED animals

1. Preparation of Slices

The rats (4 RESTRICTED and 3 OPEN) were killed by decapitation. The brain of each animal was removed within two minutes of decapitation. After the brains were removed, each brain was hemisected and the left side was isolated for use in the experiment. The hippocampus was carefully removed from the brain tissue; close attention was paid to avoid any direct contact among the dissecting instruments and the hippocampal tissue. The hippocampus was then pinned to an agar stage and placed (submerged in artificial cerebrospinal fluid) in the slicing chamber of a rotoslicer. The hippocampi were sliced were along the transverse plane, parallel to the lamellae, producing slices that were 500um thick.

Generally, 8-10 slices were obtained from each hippocampus. The slices were transferred to an incubation chamber that contained aCSF maintained at a temperature of 30 degrees celsius. This first incubation lasted one hour and the aCSF had a magnesium concentration of 1 mM. The rest of the aCSF consisted of (in mM) NaCl, 124; KCl, 5.0; NaH_2PO_4 1.25; $CaCl_2$, 2.0; $NaHCO_3$, 22.0; $C_6H_{12}O_6$, 10; (pH=7.4). The second one-hour incubation was in the same solution except for

a lower (.1 mM) magnesium concentration. The recording solution was the same as that used for the second one-hour incubation solution. Both incubating and recording solutions were aerated with a 5% CO_2 and 95% O_2 mixture of gases.

2. Recording and Stimulation

After the second one-hour incubation period in the low magnesium aCSF solution, a single slice was transferred to the recording chamber. The slice was suspended within the oxygenated aCSF by placing it on a metal mesh stage with a small piece of nylon mesh over it. The aCSF was infused at a rate of 2 ml/minute. The slice remained in this position for the duration of the stimulating-recording session for that slice. When recording was complete, the slice was removed from the chamber and a new slice was selected for the next session.

A dissection microscope was used to position the stimulating and recording electrodes accurately. A bipolar stimulating electrode, insulated except at the tips, was placed in the Schaffer collateral pathway in the CA2 region. The extra-cellular recording was accomplished with a glass micropipette that was filled with a saline solution and had a resistance of less than 10 M ohms. The recording pipette was positioned over the radiatum layer (between the pyramidal layer and the molecular layer) of the CA1 region of the hippocampus.

Stimuli were produced using a Grass S88 stimulation system and passed into the stimulating electrode. Stimulus parameters were: Duration = .2 ms; Frequency = .2 Hz; Intensity = 5V - 10V. Duration and frequency of the stimulation used was the same for all slices but the intensity varied with each slice (see below). Each electrically evoked extra-cellular field potential that was recorded was monitored visually on a Tektronix Model 5113 storage oscilloscope. A Grass AM8 Audio monitor was also used to monitor each pulse.

3. Experimental Procedures

With the hippocampal slice in aCSF, a field potential was first recorded and a population spike observed. Then the stimulus voltage (intensity) was adjusted to obtain the maximal amplitude of the EPSP and population spike following each individual pulse. Next, the stimulus intensity was adjusted so that the amplitude of the EPSP and the population spike was half the maximal amplitude. Once this adjustment was made, the stimulus intensity was held constant for that slice. The population spike disappears if the quisquilate and kainate receptors of the hippocampus are blocked because there are not enough EPSPs to elicit an action potential. However, even with the quisquilate and kainate receptors blocked, extra-cellular EPSP can be maintained and recorded. So, for this experiment, the EPSP (mV/ms) was monitored rather than the population spike.

After 10 mins of stimulating the slice and recording the slope of the EPSP, a solution of 6,7-dinitroquinoxaline-2,3-dione (DNQX) in aCSF (DNQX concentration 10 uM) was infused into the recording chamber. DNQX blocks quisquilate and kainate receptors, but not NMDA receptors. Stimulating and recording of the slice continued for 15-20 mins in the presence of DNQX. Then, the solution of DNQX was stoppered, and a solution of aCSF and AP5 (AP5 concentration 50 uM) was infused into the recording chamber. Stimulating and recording continued as before until the EPSP slope recorded approached zero and remained stable.

The dependent measure was the percentage change in the slope of the EPSP after the addition of AP5. This percentage change is indicates the contribution of the NMDA receptor to the slope of the recorded EPSP.

IV. **Data Analysis**

The dishabituation score used in the following statistical tests was derived by subtracting the number of seconds spent in the approach area during trial 3 from the number of seconds spent in the approach area in trial 4 (Diff43). In general, a positive score would indicate that an animal dishabituated and a negative score would indicate that an animal did not dishabituate.

Accordingly, the Wilcoxon Signed-Ranks test was used to determine whether or not a single group of animals

dishabituated during a test. In performing the Wilcoxon, the absolute value of each animal's dishabituation score was ranked from highest to lowest, then the sign (positive or negative) of the difference was noted for each score. All positive ranks were summed. The sum of the positive ranks constituted the U+ to be compared to the critical U+ for determining the probability that there were more and greater positively ranked differences than negatively ranked differences for a given group of animals. In this way both the direction and the magnitude of the change from trial 3 to trial 4 was taken into account.

Before proceeding with the Wilcoxon Signed-Rank tests for dishabituation, I performed an ANOVA on the ranks of the difference scores of all the animals to test for effects of sex, litter, test, amd rearing condition. The only significant sources of variability were test and rearing condition. In addition, post hoc analyses were conducted on the ranked difference scores in an effort to address the problem of repeated independent tests performed on the same animals when I re-tested RESTRICTED animals.

The results of a Tukey's Studentized Range test (a=.05; critical value of studentized range = 3.7; MSD=17.312) revealed that it is unlikely that, where animals were re-tested, the differences reported below are due to repeated independent tests on the same group of animals.

Where appropriate, I also performed $\underline{t}$ tests to compare

mean dishabituation scores between groups.

CHAPTER III

RESULTS

I. Behavioral Tests

A. Test for Allocentric Responding to Cues on the Arena Wall

The median Diff43 for the OPEN animals was 37.2 seconds (range = 80.63). OPEN animals dishabituated on the fourth trial (responded allocentrically) (Wilcoxon, n = 24, $\bar{x}$ DIFF43 = 41.0, s.d. = 22.7, $U_+ = 300$, $p < .05$) and RESTRICTED animals did not (Wilcoxon, n = 24, x DIFF43= -5.9, s.d. = 9.3, $U_+ = 16.5$, $p > .05$) (Fig. 5A). The mean dishabituation score for the OPEN animals was significantly greater than the RESTRICTED animals' (n = 24, $t = 1.98$, $p < .05$).

B. Test for Allocentric ResZponding to Cues on the Floor

The median Diff43 for the RESTRICTED group tested for allocentric behavior when provided with a visual cue on the arena floor was -7.7, range = 63.13. Mean Diff43= -4.8, s.d. = 11.93. The RESTRICTED animals did not respond allocentrically in this situation (Wilcoxon, n = 24, $U_+ = 44$, $p > .05$) (Fig. 5B).

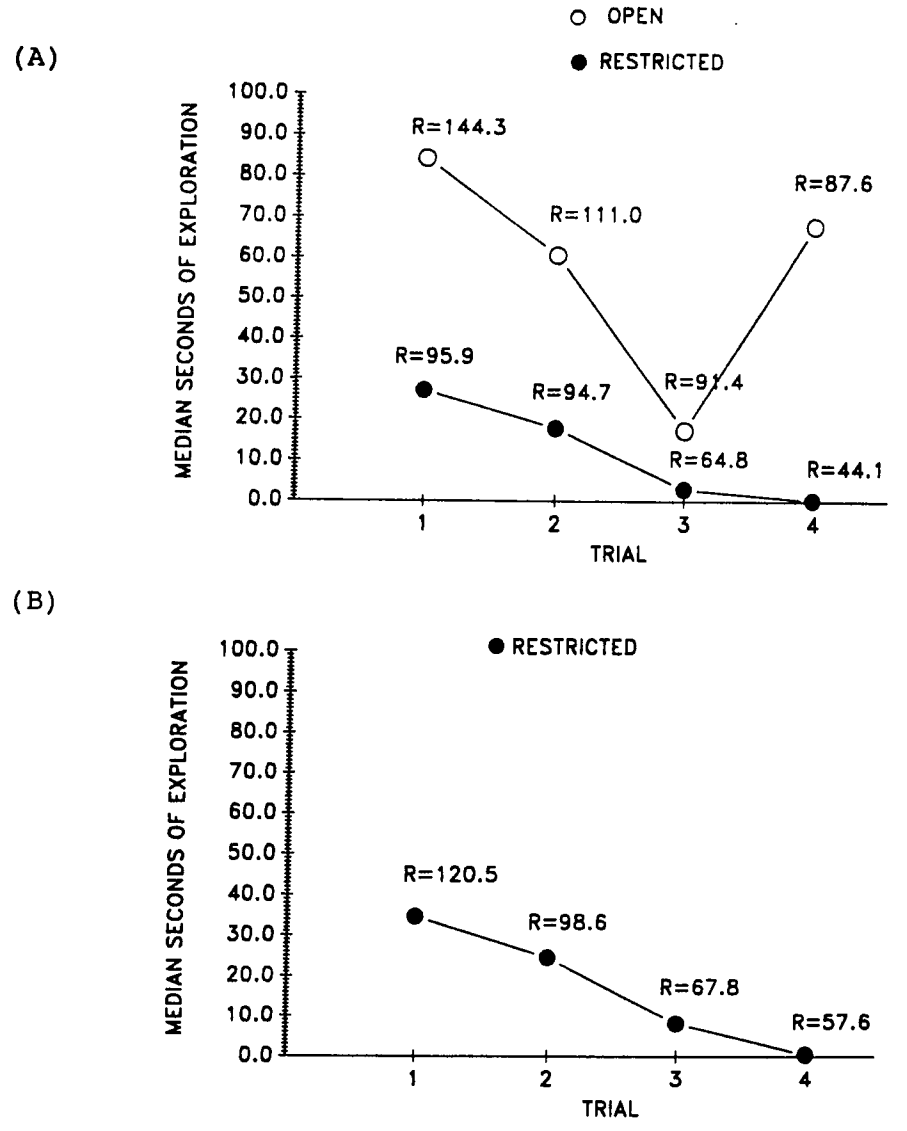

Figure 5. (A) Allocentric responding with landmarks on the walls of the arena. (B) Allocentric responding with landmarks on the floor of the arena. R= range.

C. Test for Egocentric Responding

The median Diff43 for the animals tested for egocentric responding was 25.8, range = 72.44. Mean Diff43 = 31.2, s.d. = 18.7. The RESTRICTED animals did respond egocentrically, that is, they dishabituated on trial 4 (Wilcoxon, n = 24, U_+ = 300, $p < .05$) (Fig.6).

D. Test for Allocentric Responding with Blocked NMDA Receptors

The median Diff43 OPEN animals injected with AP5 during the test for allocentric responding was -3.9, range = 24.6. Mean Diff43= -6.85, s.d.= 8.95. The OPEN animals injected with artificial cerebrospinal fluid (aCSF) + AP5 did not dishabituate on trial 4 (Wilcoxon, n = 6, U_+= 0, $p > .05$). The median Diff43 for the OPEN reared AP5-injected animals was 13.8, range = 35.5. Mean Diff43 = 12.5, s.d. = 5.6. The OPEN aCSF-injected animals did dishabituate on trial 4 (Wilcoxon, n = 5, U_+= 15, $p < .05$) (Fig.7). The mean dishabituation score for the AP5-injected animals was lower than the mean dishabituation score for the aCSF-injected group (n = 11, t = 2.26, $p < .05$).

From visual inspection of Figure 5A, the RESTRICTED animals appear to spend much less time around the objects on the first trial than do the OPEN animals. It is possible that initial level of exploration (in trial 1) is correlated with dishabituation of exploration during the fourth trial of the tests the rats were subjected to. This might indicate that rearing condition affected level of exploration rather than

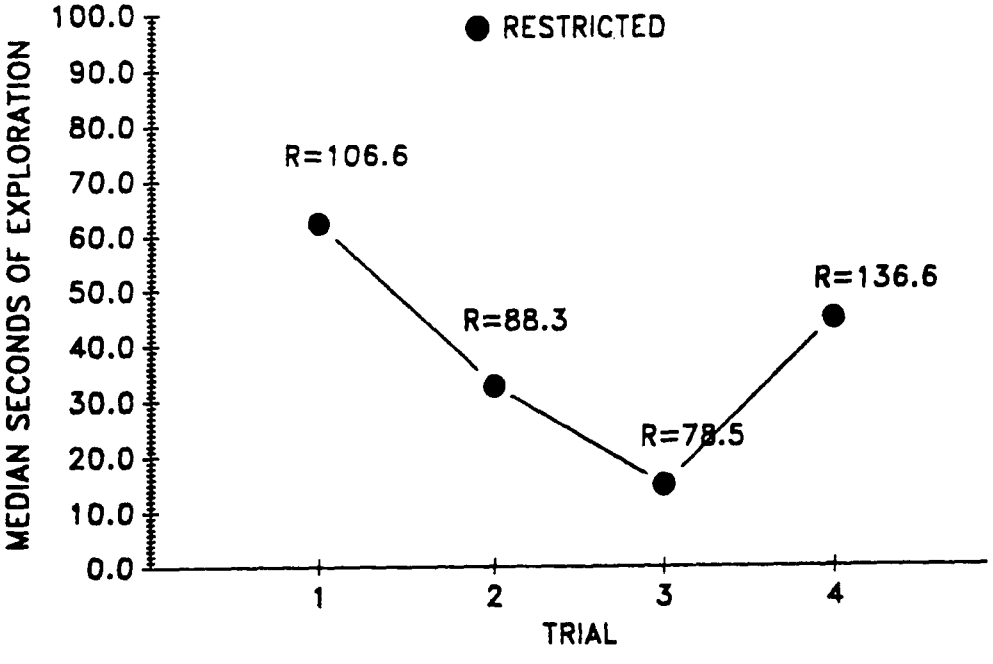

Figure 6. Results of test for egocentric responding in RESTRICTED animals. R=range.

spatial responding; that is, if RESTRICTED animals did not spend as much time around the objects as OPEN animals, they may not have learned enough about the objects to dishabituate on the fourth trial. In order to address this issue, several statistical comparisons were made. First, the mean number of seconds of exploration during trial 1 for the RESTRICTED animals in the first test for allocentric responding (n=24; $\bar{x}$=39.7 secs; sd=26.8) was compared to the corresponding value for the OPEN animals in the first test (n=24; $\bar{x}$=83.3 secs; sd=36.5) with a t-test for independent samples. The values were not different for the two groups (a=.05; t_{crit}=1.645; t_{obs}=1.420). [The same comparison was performed using the Wilcoxon Signed Ranks test for independent samples and it too revealed no statistical difference between the two groups; n=48, U^{+}=157, p>.05.]

To further substantiate the conclusion that amount of time spent exploring the objects during trial 1 was not correlated with dishabituation in a given test, exploration in RESTRICTED (n=24; $\bar{x}$=39.7 secs; sd=26.8) and aCSF OPEN animals (n=5; $\bar{x}$=42.5 secs; sd=30.24) was compared for the first trial of allocentric responding was compared. There was no difference in exploration during the first trial, yet the OPEN-aCSF animals dishabituated and the RESTRICTED animals did not in what was essentially the same test. In addition, there was no difference in exploration during trial 1 between the OPEN animals injected with AP5+aCSF (n=6; x=37.7; sd=23.06)

and the OPEN animals injected with aCSF alone, although the aCSF animals dishabituated and the AP5+aCSF animals did not.

Finally, if the RESTRICTED animals did not dishabituate on trial 4 in the allocentric tests because they did not spend enough time exploring, one would predict that they would not exhibit habituate between trial 1 and trial 3. However, Wilcoxon Signed-Rank tests for habituation based on the difference between exploration during trial 3 and trial 1 (DIFF31) indicate that all groups tested in this study habituated between trial 1 and trial 3 (for all tests a=.05, Wilcoxon: First Allo Test, OPEN n=24, U^+=3; RESTRICTED n=24, U^+=9; Second Allo Test, RESTRICTED n=24, U^+=15; Ego Test, RESTRICTED n=24, U^+=10; AP5 vs aCSF Allo Test, AP5 n=6, U^+=0; aCSF n=5, U^+=0).

Together, these results show that the amount of time spent in the approach area during trial 1 does not predict the presence or absence of dishabituation during trial 4.

D. TCP Binding Analysis

1. NMDA Receptor Number

The OPEN animals (n = 8, $\bar{x}$ B_{max} = 2.0 pmol/mg, s.e. = .32) and the RESTRICTED animals (n = 8, $\bar{x}$ B_{max} = 1.95, s.e. = .21) did not differ in amount of NMDA receptor binding under equilibrium conditions (Table 1).

2. NMDA Receptor Response to NMDA and Glycine

The OPEN animals (n = 4, $\bar{x}$ EC_{50} = 1.1uM, s.e. = .06) and

the RESTRICTED animals (n = 4, $\bar{x}$ EC_{50} = 1.1, s.e. = .06) did not differ in terms of the effectiveness of NMDA at opening the magnesium channel and facilitating TCP binding. Nor did they differ in the effectiveness of glycine at activating the channel to facilitate TCP binding (OPEN n = 4, $\bar{x}$ EC_{50} = .10uM, s.e. = .03; RESTRICTED n = 4, $\bar{x}$ EC_{50} = .09uM, s.e. = .02) (Table 1).

E. NMDA Receptor Activity in vitro

The preliminary results indicate that the NMDA receptors from an OPEN animal contributed a mean of 27% of the EPSP slope in the OPEN animals (Fig. 9A). In contrast, the same procedure performed on hippocampal slices from RESTRICTED animals indicated that the NMDA receptors were only contributing a mean of 7.8% to the EPSP (Fig. 9B).

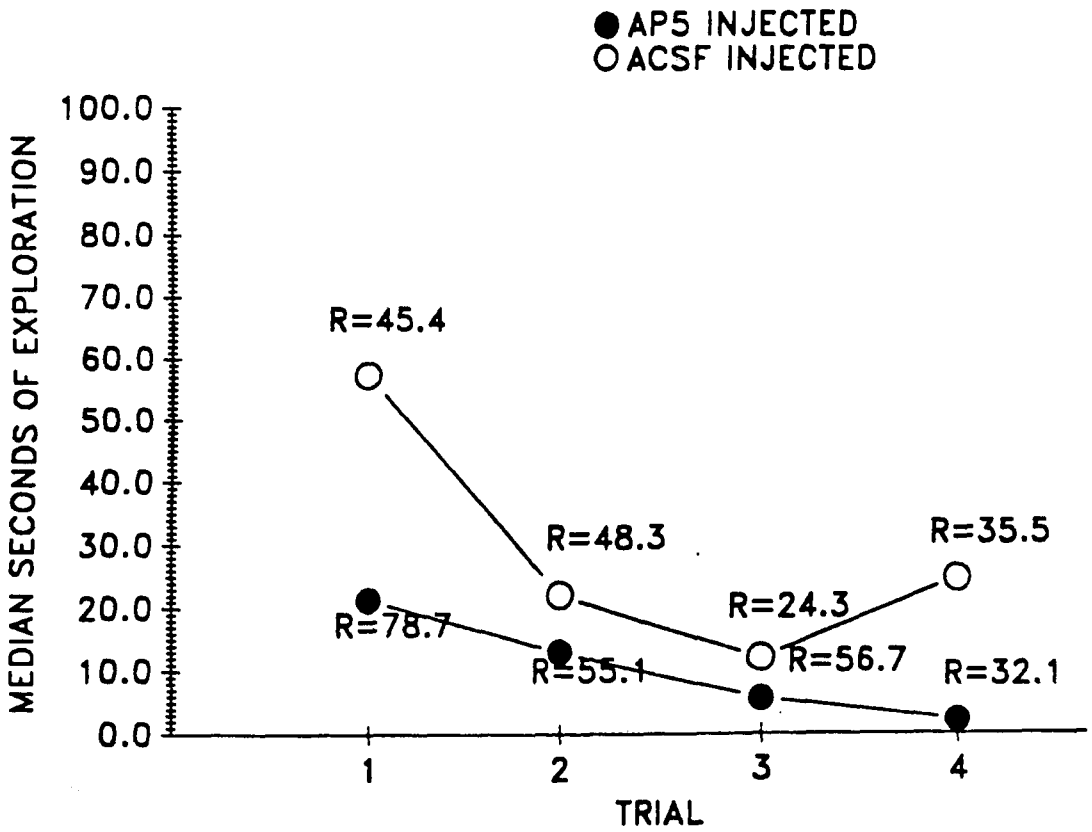

Figure 7. Results of test for allocentric responding in OPEN animals injected with AP5 or ACSF. R=range.

Table 1. Analysis of kinetic membrane binding experiments using radioactive TCP.

Kd=apparent dissociation rate/apparent association rate; EC_{50} = effective concentration; B_{max} maximum binding.

Equilibrium		Non-Equilibrium	
Restricted		Restricted	
TCP Kd	TCP Bmax	Glycine EC50	NMDA EC50
(nM)	(pmol/mg)	(uM)	(uM)
5.6. s.d.= .6	1.95 s.d.= .21	.10 s.d.= .03	1.1 s.d.= .6
Open		Open	
4.7 s.d.= .6	2.0 s.d.= .32	.09 s.d.= .02	1.1 s.d.= .6

*No difference is statistically different

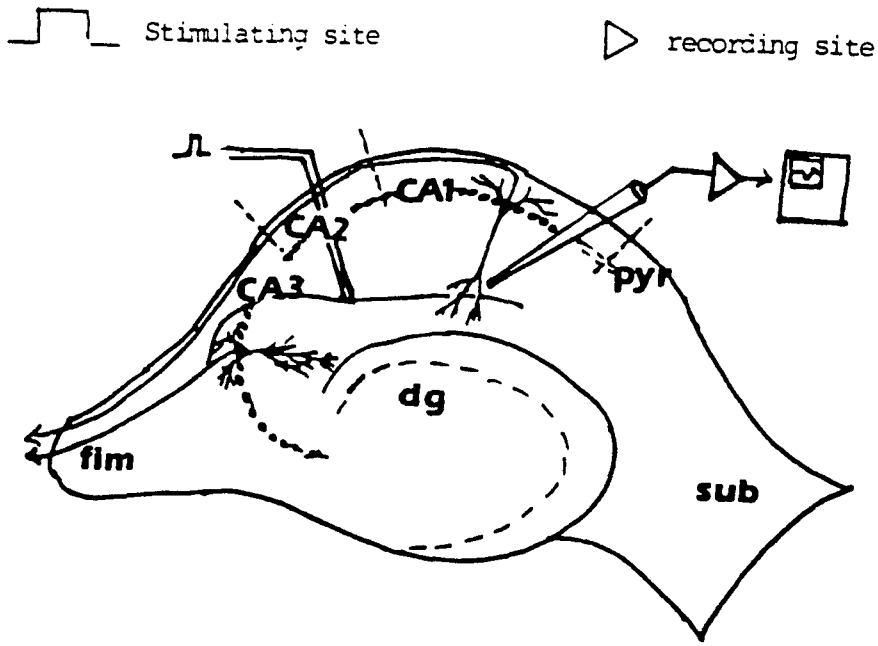

Figure 8. Stimulating and recording sites in the rat hippocampus slice preparation.

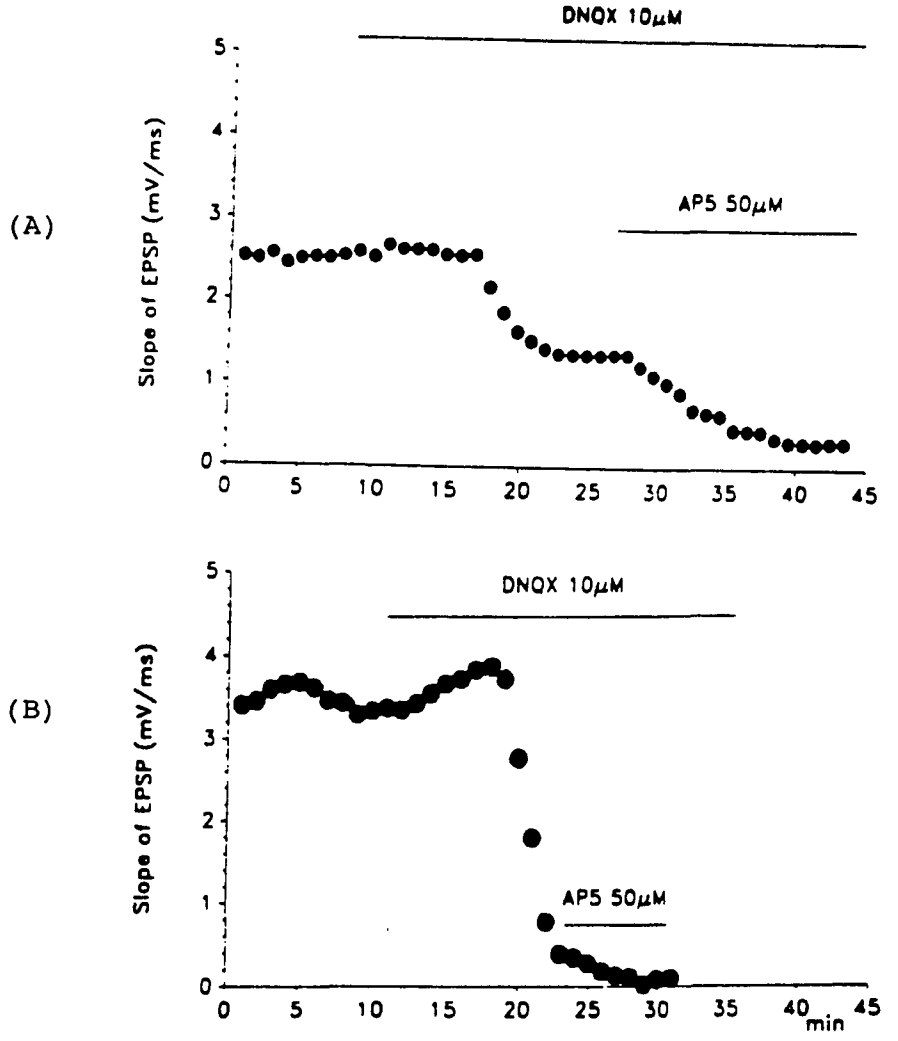

Figure 9. Change in the slope of the EPSP after blocking quisquilate, kainate, and NMDA receptors in (A) OPEN and (B) RESTRICTED animals. Note that in the RESTRICTED animals (B) the NMDA receptors contribute very little to the slope of the EPSP. These graphs show results from one slice of one animal in each condition and are representative of the other animals used in the study.

CHAPTER IV

GENERAL DISCUSSION

The results show that RESTRICTED rearing either prevented or delayed the development of allocentric responding but did not affect the development of egocentric responding. The fact that the RESTRICTED animals did not respond allocentrically when the stable cue was on the floor of the arena indicated that their failure to exhibit allocentric behavior in the first test was probably not due to an inability to see the distal cues because of near-sightedness. These findings support and extend other work with hamsters (Tomlinson, 1990) and human infants (Bai and Bertenthal, 1990) suggesting that some aspect of exploratory experience may be important for the development of allocentric behavior. The question of what specific experiences are required for the normal development of allocentric behavior remains unclear, although apparently the opportunity to explore a large space and the presence of siblings and objects is not enough.

O'Keefe and Nadel (1978) pointed out that in a changing environment, mapping or placing strategies are relatively ineffective and that egocentric based strategies tend to dominate. While a more systematic, finer-grained analysis of

different aspects of the rearing conditions is needed, the results of this study do suggest that one aspect of exploratory experience that is important for the development of allocentric behavior is experience with stable frameworks of landmarks.

One of the main differences between the two rearing conditions was access to stable visual frameworks. Neither the RESTRICTED nor the OPEN animals in this study were restricted in movement, nor were they deprived of different wavelengths of light or patterned visual stimulation. However, the plastic objects in both conditions were moveable and casual observation of the OPEN animals indicates that during play, the rats regularly moved the objects around the cage. Therefore, the main difference between the two conditions was that the RESTRICTED animals did not have access to a stable visual framework outside the cage as the OPEN animals did. This restricted access to stable visual landmarks may have provided the kind of changing environment in which O'Keefe and Nadel (1978) suggest egocentric behavior dominates. The rats in the OPEN condition had access to a stable framework of landmarks provided by the ceiling and walls of the colony room and the RESTRICTED animals did not. Perhaps this was enough for the normal development of allocentric responding. While the findings from this project are suggestive, they do not provide conclusive evidence that it was the access to landmarks that made the difference in the

two groups of animals. More detailed research, including more systematic analysis of object movement in the cage, is required to draw conclusions about the nature of the particular sensory stimulation that is required for allocentric behavioral development.

For instance, if it is the exposure to stable landmarks and not something else about the chance to see outside of the cage that is contributing to the behavioral deficit observed in the RESTRICTED animals, then fixing the colored objects to the floor of the RESTRICTED rearing cage should lead to the development of allocentric behavior in these animals. Similarly, patterns on the wall of the RESTRICTED rearing cage might also lead to the development of allocentric responding in the RESTRICTED animals.

Questions remain regarding the nature of the behavioral difference between the two groups of animals. For instance, no data were collected to determine whether the RESTRICTED animals would exhibit allocentric behavior spontaneously at some later time in development or if they were allowed to live in a clear cage for a time after 50 days in the opaque cage. It would also be informative to test animals from the two rearing conditions at different ages before 50 days old to see how soon behavioral differences become apparent. Additionally, since allocentric behavior depends on the use of configurations of landmarks, it is possible that the lack of allocentric responding in the RESTRICTED animals is

symptomatic of a more general difference, such as difficulty using stimulus configurations (Sutherland and Rudy, 1989). For instance, if there is a more general kind of deficit, RESTRICTED rats would be worse than OPEN rats at other kinds of tasks, such as using a light-tone combination to predict a food reward (Sutherland and Rudy (1989).

When the hippocampi of animals reared in clear cages were administered AP5, those animals did not perform allocentrically in the habituation paradigm, although aCSF-injected control animals did. This result extends similar findings from AP5 injection studies with the Morris water maze (Morris, 1990) to the habituation paradigm and confirms that functioning NMDA receptors in the hippocampus are important in some way for behavior based on visual landmarks. In addition, the fact that rats with blocked NMDA receptors did not respond allocentrically suggests that the rats reared in the RESTRICTED condition did not exhibit allocentric behavior because their hippocampal NMDA receptors were different in number or functioning from those of rats in the OPEN condition.

In light of the results from the AP5 injection experiment, it is perhaps surprising that the results of the [^{3}H] TCP binding analyses did not reveal a difference between the OPEN and RESTRICTED animals in hippocampal NMDA receptor number, or a difference in how effective NMDA and glycine were at opening the Ca^{++} ion channel to allow [^{3}H] TCP binding to

occur. However, the kinetic membrane analyses performed do not exhaust the range of possible differences between the NMDA receptors in the RESTRICTED and the OPEN animals.

The NMDA receptor is complex (Cotman et al. 1989) and other aspects of its structure such as the structure of the calcium channel could have been affected without making a difference in the binding experiments conducted. In addition, there may be nothing wrong with the NMDA receptor itself, but the RESTRICTED animals may have lower levels of the amino acid glycine, a potentiator of the NMDA receptor site (Bonhaus et al. 1989). Since the binding studies only addressed two aspects of the NMDA receptor it is not possible to tell exactly what may be different about them in the RESTRICTED animals. Indeed, without any further investigation, only the finding that AP5-injected animals do not behave allocentrically justifies the supposition that hippocampal NMDA receptors were important for the behavioral difference between the OPEN and RESTRICTED animals. At best that is indirect evidence that RESTRICTED animals' NMDA receptors developed differently from the OPEN animals'.

The results up to this point allow me to rule out the possibility that the behavioral difference between the two groups was due either to a difference in receptor number or to a difference in glutamate and glycine effectiveness at opening the calcium channel. One limitation of the TCP binding studies is that they only permit one to draw

conclusions about the receptors on membranes disassociated from the rest of the post-synaptic cell and do not permit any conclusions about how the receptors would respond to stimulation.

The next logical step was to try to discover whether or not the hippocampal NMDA receptors of RESTRICTED animals actually respond differently from those of OPEN animals to stimulation. Although more data are required to draw a firm conclusion, the preliminary evidence gathered indicates that some aspect of NMDA receptor functioning is definitely altered by the RESTRICTED rearing condition. Therefore, the physiological difference between the RESTRICTED and the OPEN animals does appear to involve the NMDA receptor although the difference does not appear to be one of receptor number or effectiveness of the bound glutamate and glycine sites at opening the calcium channel.

More work will be necessary to understand exactly what is different about the RESTRICTED animals' NMDA receptors. For example, RESTRICTED rearing may have altered the calcium channel or some aspect of the biochemical cascade. Further, the difference may not be in the NMDA receptor itself, but may simply involve a lower level of glycine in the RESTRICTED animals. Additionally, since it is highly likely that other neurotransmitter systems are involved in the development of spatial behavior it would also be important to assay for differences in number and functioning of receptors for

acetylcholine and norepinephrine.

It is important to emphasize that the goal of this research was not to show that only the hippocampus is involved in spatial behavior or only the NMDA receptors are important. The glutamate transmitter system is not the only one that has been studied with regard to spatial behavior. Olton et al. (1985) performed a study in which young and aged rats were tested in three spatial discriminations that varied a great deal in the extent to which flexible responding was required. Older rats were less able to perform all three of these tasks than the younger rats. In addition, the group also looked at biochemical differences between the younger and older rats. They found no correlation between age and choline concentration in the brain or between maze performance and choline content in the brain.

However, Ingram et al., (1981) found a significant correlation between individual maze performance and acetylcholine transferase activity in the hippocampus of aged rats. Further, Meck et al. (1988) report that male albino rats exposed to choline chloride supplementation prenatally and postnatally showed more accurate performance in both working and reference memory components of a 12-arm and 18-arm-radial-arm-maze task. They concluded that this difference was not due to different response strategies but to long-term enhancement in the capacity and precision of spatial memory In addition, Meck and Williams (1989) assesed the dietary

effects of choline provided prenatally (to the mother) and postnatally (given directly to the stomach of the newborn pups) on spatial performance was assessed (Meck and Williams, 1989). Their data show that perinatal choline supplementation causes (a) long-term facilitative effects on working and reference memory components of a 12-arm radial-arm-maze task and (b) increased muscarinic receptor density and increased choline acetyltransferase levels in the hippocampus and frontal cortex of adult animals. The ChAT to QNB ratio in the hippocampus is highly correlated with working memory errors and the ChAT to QNB ratio in the frontal cortex is highly correlated with reference memory errors.

The role of norepinephrine in spatial behavior was studied using systemic injections of bretylium in addition to injections of norepinephrine into hippocampally lesioned animals (Maier, Ryan, and Isaacson, 1990). Bretylium is an adrenergic blocking agent that inhibits the release of peripheral norepinephrine (NE). Hippocampally lesioned animals treated with saline were severely impaired on the Morris water maze. The adrenergic treatment produced enhanced performances in the rats with hippocampal lesions, although the treated animals were still impaired, compared to non-lesioned controls. Thus norepinephrine, as well as acetylcholine, has been implicated in spatial behavior. Obviously, information on the role of these other neurotransmitter systems will need to be integrated with the information

being obtained on the NMDA receptor system to get a complete picture of the neurophysiological changes that take place during the development of spatial behavior.

Summary

In conclusion, the behavioral results from this project indicate that the RESTRICTED rearing condition delayed or prevented the development of allocentric behavior. Further, the data from the AP5-injection experiment in conjunction with published experiments on the effects of hippocampal lesions Green, 1964; Alessandri, et al., 1978; Cotman, et al.,1989; Deyo & Conner, 1989) and other AP5-injection experiments (Morris, et al., 1986; Morris, 1989) support the hypothesis that the hippocampal NMDA receptors in RESTRICTED animals were either fewer in number or functioned differently from those of the OPEN animals. The TCP binding studies rule out the possibility that RESTRICTED animals have fewer hippocampal NMDA receptors and the possibility that NMDA and glycine were ineffective at opening the calcium channel of the NMDA receptors in the RESTRICTED animals. Preliminary evidence from hippocampal slice preparations of RESTRICTED and OPEN animals suggests that the NMDA receptors of RESTRICTED animals do not respond to stimulation in the same way that those of OPEN animals do. This may be because the RESTRICTED animals' NMDA calcium channels are defective or some other aspect of the biochemical cascade involved in receptor functioning is

defective or because RESTRICTED have lower endogenous glycine levels.

Taken together, these data lead me to conclude (provisional on more data from the hippocampal slice preparation) that the RESTRICTED rearing condition altered the development of the hippocampal NMDA receptors in those rats so that they did not respond allocentrically to visual landmarks.

Because the genetic constitution of the rats was held constant while their experience differed, this study of isogenic rats has much in common with the design of identical twin adoption studies traditionally performed by developmental psychologists to ascertain what aspects of behavior are under genetic influence and what aspects are not (Wilson, 1934; Taubman, 1976; Loehlin, 1982; Plomin, 1986). The classic interpretation of these studies is that characteristics shared by identical twins reared apart are genetically determined and characteristics that distinguish them are environmentally determined (Plomin, 1981, pp. 271-273). This approach to understanding the cause of characteristics is based on the assumption that genes and environment act independently, albeit in concert, to produce combinations of characteristics.

This dichotomous approach to understanding how organisms come to possess different characteristics has a long history of criticism (Kuo, 1967; Lehrman, 1953; Gottlieb, 1983; Oyama, 1985; Johnston, 1987; Ho, 1984; Schneirla, 1966). The primary

thrust of these criticisms of the dichotomous view of development is that within organisms during development there are always many factors contributing simultaneously to the development of any characteristic in such a truly cooperative manner that it is impossible to assign some characteristics a genetic origin and some an environmental one. Further, the interactionists argue that the dichotomous approach makes no headway toward actually understanding the mechanisms by which organisms develop, and may in fact impede understanding by appearing to provide explanations when in fact it does not (Kuo, 1967; Lehrman, 1953; Gottlieb, 1987; Oyama, 1985; Johnston, 1987; Ho, 1984).

Grounded in the tradition of the interactionist approach, this project with isogenic rats may have important implications for the way researchers think about developmental processes in general. However, the fact that these rats had identical genetic constitutions allows me to make an important point concerning developmental mechanisms. As is the case for twin studies, the dichotomous interpretation of this study is that allocentric spatial behavior is experientially rather than genetically determined; since DNA constitution was held constant for the two groups of rats and the environments were different, differences in behavior cannot be attributed to differences in genes.

However, the interactionist approach would lead to another interpretation of these results. To the extent that

many structural changes that take place in the nervous system require some new protein production (Rose, 1989), one mechanism of spatial behavior development may involve an effect of sensory stimulation on nucleic acid activity in cells of the central nervous system. In this way, animals with the same genetic constitution would potentially develop different nervous systems and different behavior. These differences would occur as a consequence of the different patterns of gene activity occurring in tandem with differences in sensory stimulation. From this perspective, if one is asking how a behavior develops, it is not appropriate to categorize the behavior as either genetically or environmentally determined because the DNA activity and the sensory stimulation are interdependent during development. Nucleic acid activity depends to some extent on sensory stimulation and the sensory stimulation that impinges on the nervous system depends to some extent on nucleic acid activity.

The idea that sensory stimulation alters nucleic acid activity is not a new one (Hyden and Egyhazi, 1962; Uphouse and Bonner, 1975 Grouse, et al. 1981). Currently, there is a literature devoted to investigating the mechanisms by which sensory stimulation alters nucleic acid activity during learning in chicks during passive avoidance training (Rose, 1990) and imprinting (Horn, 1990). Briefly, there is evidence that a key event following sensory stimulation (such as exposure to a passive avoidance task) is a change in the

phosphorylation of a protein known as B50, F1, or GAP 43 (Kaczmarek, 1987; Routtenberg, 1985; Skene, 1989). Chiarugi, et al. (1989) have suggested that this phosphorylation change may be associated with an increase in calcium influx into synapses and that the increased calcium levels may interact with the gene families c-jun and c-fos. As a consequence of the activation of these genes, glycoproteins are synthesized and inserted into pre- and post-synaptic membranes to produce a lasting change in the synaptic structure associated with long-term memory (Burchuladze et al. 1990). This hypothesis is based on the idea that changes in the nervous system accompanying specific kinds of learning are due to changes in nucleic acid activity.

Isaacson (personal communication) has criticized this approach, suggesting that the structural changes that take place during learning occur too quickly to involve the synthesis of new proteins (Fig. 10). Indeed, the synthesis of new proteins is thought to take several hours (Thomas and Meizel, 1989) and Burchuladze et al. (1990) report that the structural changes they observe occur as early as 10 minutes after the passive avoidance training. Isaacson suggests that stereochemical changes are more likely responsible for the structural changes observed because they can occur very quickly.

However, Horn (1990) indicates that the changes in the chick brain he observes after imprinting appear to occur three

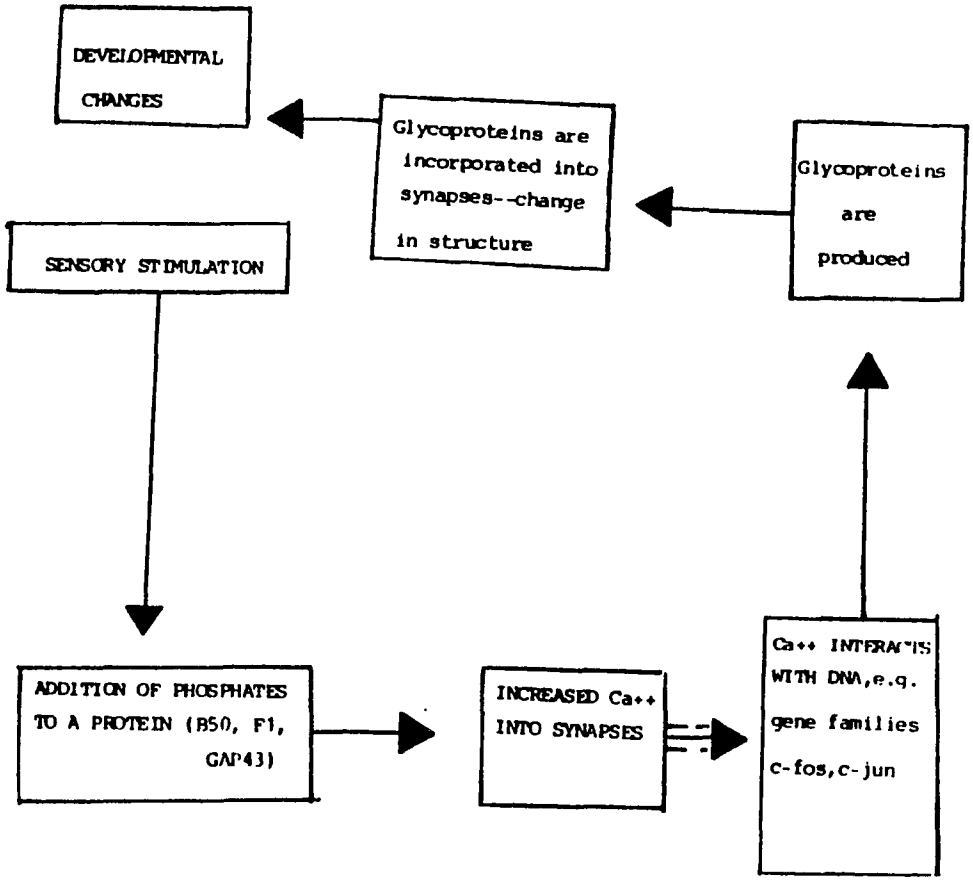

Figure 10. Theoretical mechanism for sensory stimulation to alter DNA activity.

hours after the imprinting training. Further, Horn and McCabe (1985) report that the change they observe following imprinting is an increase in number of NMDA receptors, which is more likely to require synthesis of new proteins, and therefore nucleic acid activity, than the phosphorylation changes that Rose (1989) reports in conjunction with passive avoidance training.

Therefore, it is possible that changes in DNA activity with differences in sensory stimulation may still be involved in some aspects of behavioral development, such as the development of spatial behavior. The rats in this study were exposed to their particular rearing conditions from birth to 50 days of age. It seems entirely possible that a mechanism such as the one Burchuladez et al. (1990) propose may be at work in this case. Fifty days is plenty of time for synthetic activity to change and the structural differences between nervous systems of animals reared in different environments can be profound (Rosenzweig, et al., 1969; Singh, et al., 1969; Juraska, et al., 1980; Renner, et al., 1987). If some structural differences between animals involve the production of more protein, then some mechanism for sensory stimulation to alter nucleic acid activity must exist.

If sensory stimulation directly or indirectly alters protein synthesis crucial for building the nervous system, it is inappropriate to ask whether the behavior that results is genetic or environmental in origin. As others have repeatedly

urged (Gottlieb, 1987, Lehrman, 1953; Schneirla, 1966) researchers interested in development might consider replacing the question "Is this behavior primarily genetic or primarily environmental in origin?" with the question "Given that a certain factor appears to play a role in the development of a behavior, by what mechanism is that factor acting to influence development?"

Of course, the existence of mechanisms for sensory stimulation to alter nucleic acid activity in the nervous system is still in question. The research reported here leads to the prediction that such mechanisms exist. The actual mechanisms still need to be uncovered. It is possible that the development of hippocampal NMDA receptors in conjunction with spatial behavior development in the rat will provide a useful mammalian model for investigating this potential mechanism of developmental change.

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