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Student's signature

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Shala N. Blue

The Role of the Hippocampus in the Development of Spatial Memory

By

Shala Blue
Master of Arts

Psychology

_____
Jocelyne Bachevalier, Ph.D.
Advisor

_____
Patricia Bauer, Ph.D.
Committee Member

_____
Joseph Manns, Ph.D.
Committee Member

Accepted:

_____
Lisa A. Tedesco, Ph.D.
Dean of the Graduate School

_____
Date

The Role of the Hippocampus in the Development of Spatial Memory

By

Shala Blue
B.S., Morgan State University, 2005

Advisor: Jocelyne Bachevalier, Ph.D.

An abstract of
A thesis submitted to the Faculty of the Graduate School of Emory University
in partial fulfillment of the requirements for the degree of
Master of Arts
in Psychology
2009

Master Thesis
Abstract

The role of the hippocampus in the development of spatial memory
By Shala Blue

Previous studies have suggested that the protracted development of certain spatial memory processes in primates may be correlated with the protracted neural maturation of the hippocampus. However, due to a lack of evidence in non-human primates regarding normal structural and functional maturation of the hippocampus, the present study investigated whether: 1) hippocampal-dependent spatial memory had a protracted development, 2) early damage to the hippocampus resulted in delayed impairments in spatial memory that correspond to the timing at which the hippocampal-dependent spatial memory abilities emerge, and 3) the effects of early damage to the hippocampus mimicked the effects of adult damage or whether significant sparing of spatial memory functions occurred after the neonatal hippocampal lesions. Rhesus macaque monkeys that received neonatal ibotenic acid lesions of the hippocampus (N=5) and controls (N=6), were tested on two versions of a VPC paradigm measuring spatial location memory and object-place associations at 8-months, 18-months and 5-6 years. Performance was evaluated throughout development and adult performance of animals with neonatal hippocampal lesions was compared to the performance of animals with adult lesions of the hippocampus, using the same paradigm, from a previous study. We found evidence for a protracted development of spatial memory abilities in normally developing rhesus monkeys with regards to both spatial location and object-place association. In addition, we found that animals with bilateral neonatal hippocampal lesions did not show a preference for novelty throughout development and that their performance as adults was similar to animals with bilateral adult lesions.

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CHAPTER 1: INTRODUCTION

The Role of the Hippocampus in the Development of Spatial Memory

Introduction

Processing information regarding one's environment for later recall is a practical ability that most humans, non-human primates, and other animals share. Navigating through one's environment is essentially dependent on an organism's spatial memory. Although it has many definitions, the most canonical meaning of spatial memory is that it refers to the process by which an organism encodes, consolidates, stores, and retrieves information it obtains through sensory experiences with its environment/surroundings.

Significance of Spatial Memory

The information gained through interaction with the environment is crucial to animals, and is essential for natural adaptation. Animals living in complex social and structural environments (i.e. rodents, birds, humans and monkeys) utilize spatial skills to solve complex problems and to perform ecologically relevant tasks, such as foraging, food storage and retrieval, locating mates, homing, migration, and navigating throughout home territories. For instance, some bird species cache food for later retrieval (Krebs et al, 1989; Sherry et al, 1992; Shettleworth, 1990) and mature male rhesus macaque monkeys leave their natal groups during breeding seasons in order to search for mates (Colvin, 1986; Hill, 1986; Sugiyama, 1976). In humans, spatial memory serves as a means to accomplish complex tasks such as driving, which for example, allows taxi drivers to effectively navigate through busy and confusing London streets using specific landmarks in order to find different locations (Maguire et al, 1997; Maguire et al, 2000).

Types of Representations in Spatial Memory

It had previously been proposed that there were different kinds of representations with regards to spatial knowledge (Nadel and Hardt, 2004; O'Keefe and Nadel, 1978). Reference frames generate spatial representation strategies that help organisms to navigate in space and that allows an organism to locate an object or thing in space. Allocentric and egocentric viewpoints are the frames of reference to which O'Keefe and Nadel (1978) refer in their cognitive map theory. Allocentric (or viewpoint independent) representation refers to spatial information provided by spatial relationship between stimuli and apart from the organism, in other words, it is the spatial information in the environment independent of the observer (i.e. the bike is on the left of the cherry tree). On the other hand, egocentric (or viewpoint dependent) representation refers to spatial information from the organism's viewpoint, thus it is the individual's perception of oneself in space (i.e. I am about 3 yards to the left of the bike). Nadel and Hardt (2004) contend that animals use by default both allocentric and egocentric strategies to navigate space. They may choose one strategy over the other or integrate the two in order to solve spatial problems (i.e. In order to get to the cherry tree I must go to the right of the bike). These spatial strategies have long been shown to be important in numerous foraging, place learning, and navigation paradigms.

Behavioral Paradigms

There have been many controlled behavioral techniques employed to understand spatial memory processes. These tasks are believed to be taxing allocentric/egocentric representations in spatial memory while at the same time providing a simple means of

assessing the mechanisms by which spatial memory may be demonstrated. In rodents, there are a series of maze learning tasks used to test spatial memory (see for review Dudchenko, 2004), which in some cases have been adapted to suit primates including humans (Bohbot et al, 2002; Overman, 1996). Some of these mazes include the Morris water maze (rodents - Morris, 1981; humans - [Invisible Sensor task] – Bohbot et al, 2002; Overman et al, 1996) the radial arm maze (rodents - Olton & Samuelson, 1976; Olton & Papas, 1979; humans- Aadland et al, 1985; Overman et al, 1996), and tasks requiring large scale environments i.e. path integration and arena task (rodents - Mittelstaedt and Mittelstaedt, 1980; monkeys - Glavis-Bloom & Bachevalier, 2006; Hampton et al, 2004; Lavenex et al, 2006; 2007b; humans - Bohbot et al, 2002; Overman et al, 1996).

Other tasks have also been utilized to test spatial relational memory and spatial working memory abilities. In rodents, non-human primates, and humans, tasks such as spatial delayed non-matching to sample (spatial DNMS: see for review Dudchenko, 2004; Curtis et al, 2004; Mahut & Moss, 1986; Weed et al, 1999; Wiig & Bilkey, 1994a) or delayed nonmatching to location (DNML: Alvarado and Bachevalier, 2005a, b; Murray & Mishkin, 1998), spatial alternation (Clark et al, 2000; Schenk, 1985; Green and Stanton, 1989), and variants of novelty preference or spontaneous novelty exploration paradigms (Mumby et al, 2002) , including versions of the visual-paired comparison task (Bachevalier and Nemanic, 2008) have been used to study various kinds of spatial memory processes as well as the neural circuitry supporting these processes.

Neural Substrates of Spatial Memory

Given that spatial memory is essential for survival and for the daily functioning of many organisms, it is important to understand what neural structures help to facilitate this kind of memory. Since the famous case of H. M. in the 1950's (Scoville & Milner, 1957), the neural mechanisms underlying memory have been vastly studied. Research has targeted the temporal lobe (the medial temporal lobe [MTL] in particular) as the site of multiple memory processes (Gaffan, 1995), including spatial memory.

The most widely explored structure within the MTL that has been viewed as essential in the execution of spatial memory tasks is the hippocampal formation, which includes the hippocampus proper [CA1-3], subiculum/parasubiculum, and the dentate gyrus (Figure 1A - Moscovitch et al, 2005, 2006; Nadel, 1991; Nadel & Hardt, 2004; O'Keefe & Nadel, 1978). However, other structures have been shown to support memory for spatial representations. These structures include the parahippocampal cortex (Buffalo et al, 2006; Ploner et al, 2000), and the entorhinal and perirhinal cortices (Fyhn et al 2004; Bilkey & Liu, 2000; Liu & Bilkey, 2001; Liu & Bilkey, 1999; Liu & Bilkey, 1998a, b, c; Ramos & Vaquero, 2005; Wiig & Bilkey, 1994a, b), which are intimately connected with the hippocampus (Lavenex & Amaral, 2000) and are thought to provide support for spatial performance. For the purpose of the current project the remaining of the discussion below will focus on the hippocampus.

The role of the hippocampus in spatial memory has received strong support from lesion (Alvarado & Bachevalier, 2005b; Astur et al, 2002; Beason-Held et al, 1999; Buckley, 2005; Moses et al, 2005; Mumby et al, 2002; Liu & Bilkey, 2001; Murray & Mishkin, 1998; Scoville & Milner, 1957), electrophysiology (see for review Bilkey, 2007; O'Keefe and Nadel, 1978) and imaging (Aggleton & Brown, 2005; Ekstrom &

Brookheimer, 2007; Maguire et al, 2000; Maguire et al, 1997;Taylor et al, 2007) studies in many species.

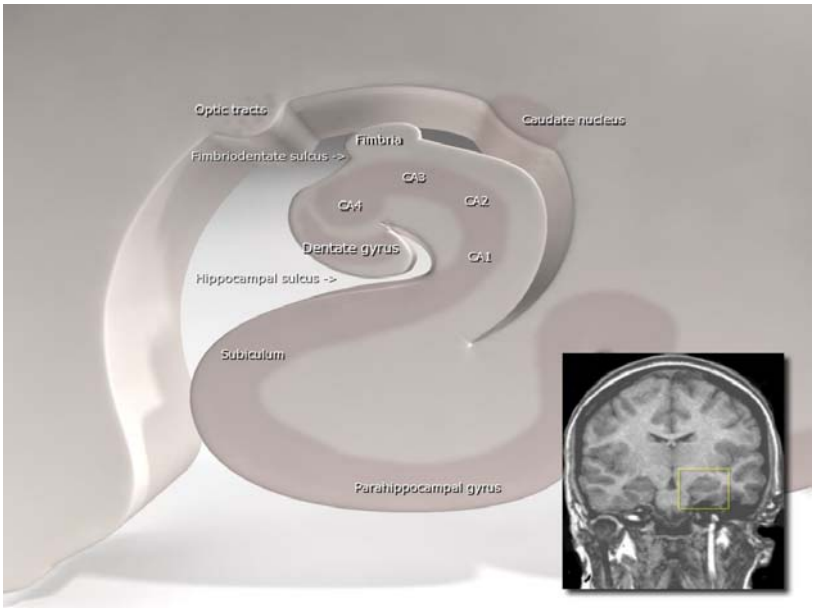
Ontogeny of Spatial Memory Processes in Primates

For a better understanding of the role of neural structures in remembering spatial representations, researchers have attempted to trace the development of this type of memory. To date, studies have assessed its developmental course in humans and in some non-human animals. Haun et al (2006a) studied the comparative ontogeny of spatial cognition in humans and non-human primates. The researchers found that adult apes in all Hominid subtypes (*Pongo, Gorilla,* and *Pan*) and nonlinguistic children (age 1) showed a preference for place strategy (in which a reward remained in a static location but hidden under different objects) in order to complete spatial tasks, whereas older linguistic children (age 3) prefer feature strategy (in which the location of the reward changes with the object under which it is hidden) in completing such tasks. In another study from the same laboratory, adult apes and preschool children (age 4) showed a preference for allocentric cues over egocentric cues in solving spatial relational strategies (Haun et al, 2006b).

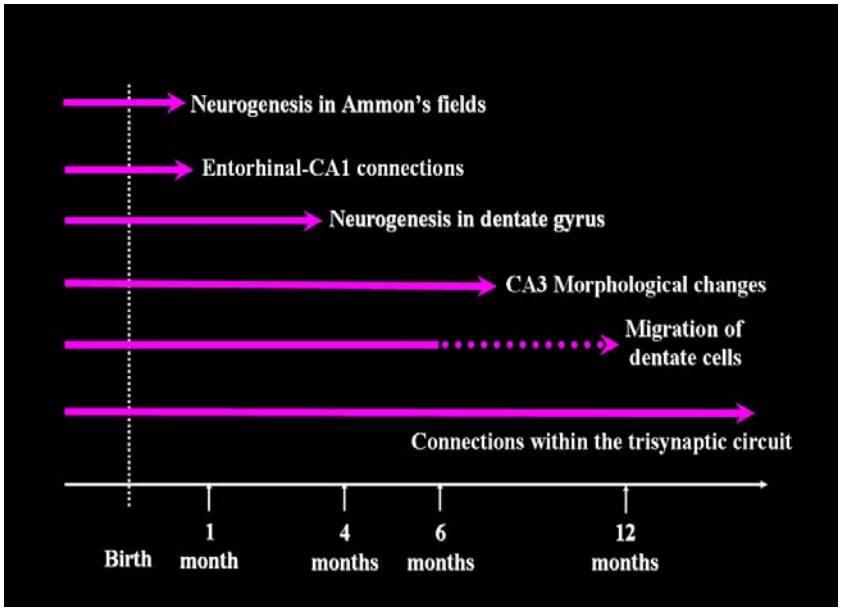
Overman et al (1996) studied the ontogeny of place learning (which taxes spatial memory by having a subject travel to and remember a particular place in order to get a reward) in humans utilizing maze learning tasks developed in rodents and previously described above. Children and adults had to navigate either through a scaled-size radial arm maze, a human-size dry Morris maze, or a large open field arena (golf course). For each task, subjects were younger children (typically under 5-7 years of age depending on

Figure 1: Neuroanatomy of the Primate Hippocampus.

Stylized coronal MRI scan and diagram of the adult primate (human) hippocampus (A). The Cornu Ammons subfields of the hippocampus are noted as CA1-CA4, and sulci are noted with an arrow (from Gaillard, 2008). Schematic timeline of the maturation of the primate (monkey) hippocampus throughout early development in months (B). Pink arrows represent the end of major developmental progression, the dotted vertical line represents birth, dotted horizontal pink line represents tapering off maturation (adapted from Seress, 2007; Seress & Ribak, 1995a, b).



A. Diagram of Human Hippocampus



B. Timeline of Maturation of Monkey Hippocampus

task) and older children (older than 5-7 years depending on task). Adult participants (older than 17 years of age) were only assessed on the radial arm maze and open field task. For the radial maze task, children under age 5 did not use algorithmic solutions (i.e. visit adjacent arms in succession) to solve the maze (only half of children over 5 and all adults used algorithms). In addition, they were impaired as compared to older children and adults in locating rewards in a forced choice version of the task in which proximal cues were absent to facilitate the use of stimulus- reward association to solve the spatial task. Thus, they were impaired compared to older children and adults when required to use only spatial relational strategies.

In the dry Morris search task, children under age 7 were not as good as the older children in learning goal locations, although none of the children used proximal cues in the maze to locate rewards. Finally, in the open field task, children under age 9 were less accurate in locating a previously visited hidden goal, although subjects of all ages were able to locating a partially visible cued goal. The data suggested that young children were not able to use spatial relational strategies to navigate through the mazes, although they were proficient in using cues to find the reward. Thus, spatial relational memory is not present until children are approximately 7 years of age. Although young children were able to (1) use spatial strategies to complete certain spatial tasks, and (2) navigate through the mazes using cues, late emergence of proficient spatial relational ability may coincide with the maturation of structures in the medial temporal lobe that are important for spatial memory.

Maturation of Neural Structures in Spatial Memory

Neuroanatomical Development of Primate Hippocampus: A review by Alvarado and Bachevalier (2000) indicated that whereas maturation of the rodent medial temporal lobe occurs almost entirely during the postnatal period, maturation of the primate medial temporal lobe, for the most part, occurs prenatally (Rakic and Nowakowski, 1981). Nevertheless, even in primates, there exists some postnatal refinement of anatomical patterns within the hippocampus until approximately 2 years of age in the monkey and until 5 years of age in humans (Lavenex et al, 2007a; Seress & Ribak, 1995a, b; Seress, 2001). In the monkey, genesis of neurons in the dentate gyrus continues throughout gestation and is approximately 80% complete at birth, but tapers off between the fourth and sixth postnatal months to a low level that may continue through adult life. In the CA fields, CA3 neurons increase in size, number, and the spines increase in complexity in the second half of the first postnatal year, and new mossy fiber synapses are formed throughout the first year. Lastly, myelination of hippocampal afferents and efferents shows substantial postnatal maturation (Seress, 1992; Seress & Ribak, 1995a, b). This postnatal development of the hippocampus is also evidenced by an increase in hippocampal volume as well as changes in the ratio of gray to white matter from birth to 1 year of age (Figure 1B).

Hippocampal Maturation and Memory Function: The question that remains to be answered is how can the protracted anatomical changes in the hippocampus predict the age at which hippocampal-dependent memory functions emerge? Although such an association between development of hippocampus and emergence of hippocampal-dependent memory processes has been demonstrated in rodents (see for review Alvarado and Bachevalier, 2000; Galea et al, 1994; Green & Stanton, 1989; Rudy & Paylor, 1988;

Rudy et al, 1987), in monkey such direct brain-behavior association is difficult to make because performance on hippocampal-dependent tasks emerges at different time points during development. Thus, incidental recognition memory processes as measured by the visual-paired comparison task is present early in infancy, whereas relational memory processes (including spatial memory) emerge just before the second year in monkeys (see for review Bachevalier, 2001). Alvarado and Bachevalier (2000) proposed that some object memory processes may be functional early in infancy and may be supported by the direct pathway linking the entorhinal to the CA1 field; a pathway which is present in infancy. By contrast, relational memory processes, such as spatial memory, may require the functional maturation of the trisynaptic pathway that seems to emerge later. This proposal has not been espoused by Lavenex and colleagues (2007a, b) who contend that the lack of data on hippocampal development in monkeys make it impossible to determine specific developmental timeline of the structural and functional maturation of the hippocampus. Hence, further studies on the normal development of the hippocampus and adjacent cortical areas are clearly needed.

Effects of Early Hippocampal Damage on Memory Processes

Monkey studies: Earlier studies using nonselective neonatal hippocampal lesions (i.e. including the parahippocampal cortex) indicated that such damage severely impacted abilities to solve several hippocampal-dependent memory tasks, such as incidental recognition memory as measured by the VPC task (Pascalis & Bachevalier, 1999) and object and spatial relation memory as measured by the transverse patterning task and the delayed nonmatching-to-location (Alvarado, Wright, & Bachevalier, 2002), respectively.

These data suggest that memory processes subserved by the hippocampus are severely impacted after early hippocampal damage and that no other structure in the brain appears to be sufficient to take over the function.

More recently, however, Lavenex et al (2007b) assessed spatial memory functions in juvenile monkeys that had received selective neurotoxic lesions of the hippocampus in the first few weeks following birth. Interestingly, these operated animals showed normal abilities to solve a spatial memory task. The data indicate that spatial relational memory was spared after neonatal hippocampal lesions and led the authors to conclude that, following early damage to the hippocampus, brain reorganization was likely to occur such that area(s) not previously important for spatial information processing may compensate for this function after neonatal hippocampal damage. Thus, the divergent results between the Alvarado et al (2002) and the Lavenex et al (2007b) studies may be accounted for by the lesion extent, which in the case of the earlier study included areas within the parahippocampal gyrus that are now known to subserve spatial memory (Bachevalier & Nemanic, 2008; Buffalo et al, 2006; Ploner et al, 2000), or by the type of spatial memory processes subserved by the different spatial memory tasks in the studies. This latter possibility is in fact supported by recent human data indicating that neonatal hippocampal damage affects some types of spatial memory tasks but not others (see below).

Human studies: Several studies have shown that early damage to the hippocampus impairs memory functions (see for review Bachevalier & Vargha-Khadem, 2005; de Haan et al, 2006; Mishkin et al, 1997; Vargha-Khadem et al, 1997). A number of amnesic patients, have been extensively studied due to their behavioral outcomes

following early selective damage to the hippocampus (see for review Vargha-Khadem et al, 1997; Gadian et al, 2000; King et al, 2002; Mishkin et al, 1997; Spiers et al, 2001; Vargha-Khadem et al, 2003). For example, two such cases, (Beth and Jon) received damage to the hippocampus very early in life - before age 4, while a third case (Kate) experienced damage to the hippocampus during school age - age 9. All individuals had an episode of anoxia-ischemia associated with either difficulty at birth (Beth), with premature birth/convulsions early in life (Jon), or with an accidental, toxic drug dose (Kate). It is important to note that while the appearance of memory deficits in Beth and Jon occurred after a specific lapse in time, not until age 5 or 6, Kate's memory impairments were immediate. These findings further emphasize a protracted maturation of the hippocampus since the later impairments associated with a nonfunctioning or abnormally functioning hippocampus in both Beth and Jon were only observable at a time when 5 and 6 year old should have been able to perform certain memory functions, thus distinctly identifying differences in mnemonic abilities from age matched normally developing children. Consequently, it is understandable for Kate's memory impairments to have immediately been observed since the maturation of the hippocampus was already near adult levels by the time the damage occurred in her brain, which probably illustrated her clear behavioral and psychological deficits as compared to school aged children. Thus, Bachevalier and Vargha-Khadem (2005) concluded that even with greater neural plasticity in infancy, no other structure could function as a substitute in the absence of a functional hippocampus.

Although all three cases mentioned above showed memory impairments, which were consistent with the finding of patients who experience adult amnesia, each were

able to develop normal language and social skills (Mishkin et al, 1997). Thus, the effects of selective early hippocampal damage were clearly specific to mnemonic functions. However, although some spatial mnemonic functions were severely impaired, others seemed to be spared and thus reiterate hippocampal - dependent and - independent spatial memory capabilities. For example, patient Jon (see for review Burgess et al, 2002), whose early damage was presented with severe atrophy of the hippocampal region with no apparent damage to adjacent cortical areas, was found to be unimpaired on recognizing visual topographical scenes, but was impaired on a number of other spatial relational tasks including object location, and a series of navigational and place learning virtual reality spatial tasks. Findings like these in humans are important because they indicate that (1) neonatal hippocampal damage result in severe memory deficits that cannot be compensated by any other brain regions, and (2) the deficits in spatial memory are reflected in only spatial tasks that involve complex navigational solutions and more complex spatial relational solutions (Burgess et al, 2002). In fact, this later assumption converges with the idea that different spatial memory tasks may tax different neural substrates (Nadel and Hardt, 2004) and that those spatial memory abilities that are dependent on an intact hippocampus cannot survive after damage to this area. These data also indicate that the divergent results in the studies of Alvarado et al (2002) and Lavenex et al (2007b) could have resulted in differences in the spatial memory processes measured by the tasks used in the two studies.

Previous and Current Investigation

To gain further knowledge on the role of the hippocampus on the development of spatial memory abilities in monkeys, our laboratory has recently designed new experiments to investigate the effects of selective neonatal hippocampal lesions on spatial memory processes across several developmental periods (Kazama et al, 2003) using modifications of the visual-paired comparison (VPC) task. These tasks were selected because of their incidental nature that permits to measure memory abilities in very young monkeys and because selective hippocampal lesions in adult monkeys are known to impair one specific type of spatial memory (object-place association) ability but not the other (spatial location). Performance of monkeys with neonatal hippocampal lesions and their sham-operated controls on these tasks was assessed at the age of 8-months and was compared to that of adult monkeys with hippocampal lesions or sham-operations (Bachevalier & Nemanic, 2008).

The initial findings of Kazama and colleagues (2003) indicated that 8-month-old sham-operated monkeys performed more poorly on both spatial memory tasks as compared to adult sham-operated animals, although they were unimpaired on a control visual object recognition memory task. The results suggested that spatial memory abilities may still be immature at this early age, presumably due to a functionally immature hippocampus. This conclusion was supported by performance of infant monkeys with neonatal hippocampal lesions when tested on the same three VPC tasks. As compared to monkeys that had received selective hippocampal lesions in adulthood, which were impaired only in object-place association task but not the spatial location task (Bachevalier & Nemanic, 2008), the monkeys that had neonatal hippocampal lesions performed as poorly as their age-matched controls on both VPC spatial tasks. Overall,

the data suggest that, although some spatial mnemonic abilities may not be fully developed early on, there may be a period between infancy and adulthood during which animals could master spatial memory tasks mediated by the hippocampus. Thus, to gain more information on the normal development of spatial memory abilities in monkeys and their dependence on a functional hippocampus, we pursued the study initiated by Kazama and colleagues (2003) and tested the same animals longitudinally at older ages.

Rationale

The current investigation aims (1) to develop a timeline of the maturation of spatial memory abilities in monkeys by assessing these abilities in normally developing animals, (2) to investigate the role of the hippocampus in the development of these spatial memory abilities by comparing normally developing animals to monkeys with neonatal lesions to the hippocampus, (3) to investigate whether hippocampal lesions produce a global deficit in spatial memory or whether the deficit occurs in some spatial tasks but not others, and (4) to determine whether neonatal hippocampal lesions will show deficits comparable to adult lesions of the hippocampus or whether compensatory mechanism may result in sparing of spatial memory abilities over time.

We hypothesized that:

1- Spatial memory abilities in the operated controls would improve with age and may emerge at different times for different spatial tasks, indicating a protracted development of spatial abilities that may coincide with the time-course of anatomical maturation of the hippocampus described by Seress (1992; 2007) and Seress and Ribak (1995a, b).

2- As with the case of Jon (Burgess et al, 2002), animals with hippocampal lesions would be impaired on some but not all spatial memory tasks, indicating a critical role of the hippocampal formation in certain spatial memory processes. But also, the impairment should emerge at an age when specific spatial abilities emerge in the control animals.

3- Neonatal lesions of the hippocampus would result in an impairment of the same magnitude as that found in the adult monkeys with the same lesions, suggesting that although plasticity of the brain during development is likely, no other neural structures can fully compensate for the spatial memory functions mediated by the hippocampus.

CHAPTER 2: METHODS

Subjects

Eleven adult (5 male and 6 female) rhesus macaque monkeys (*Macaca mulatta*) were used in this investigation. A total of 12 monkeys were tested, however, one male monkey was dropped from the investigation due to lack of evidence of lesion extent. All monkeys received neonatal surgical brain procedures at 10-15 days of age. Five infants (Group H-ibo, 3 males and 2 females) received neurotoxic lesions of the hippocampus and six infants (Group C, 3 males and 3 females) received sham operations. The data from these animals were compared with those of animals with adult hippocampus and sham operations, tested in the same behavioral paradigm, in a previous study (Bachevalier and Nemanic, 2008).

Animals were individually housed, and, because of the nature of the behavioral tasks, were not food deprived during testing. The animals received a diet of monkey biscuits (Lab Diet #5045, PMI Nutrition International Inc., Brentwood, MO), fresh fruit and vegetable enrichment, and water was give ad libitum. At different developmental time points each of these animals had been tested on a number of behavioral paradigms including object VPC and delayed nonmatching-to-sample (DNMS). These animals also have a history of chair training which includes viewing images in a darkened room.

All procedures used in this study were approved by the Animal Care and Use Committee of Emory University. All surgeries and experimental testing of animals as infants (8-months) and juveniles (18-months) were previously conducted at the University of Texas at Houston. All previous procedures were approved by the Institutional Animal Care and Use Committee at the University of Texas at Houston, and

were conformed to the NIH Guide for the Care and Use of Laboratory Animals (DHHS publication 85-23, 1985).

Neuroimaging and Surgical Procedure

All surgeries were performed under deep anesthesia using aseptic conditions. Prior to surgery, Magnetic Resonance Images (MRI) were obtained using a GE Signa 1.5T Echo Speed scanner (GE Medical Systems, Milwaukee, WI). Two sequences were performed for each subject: a T1-weighted high resolution scan and a Fluid Attenuated Inversion Recovery (FLAIR) scan. The high resolution images were used to select and calculate the three dimensional coordinates (anterior-posterior, medial-lateral, and dorsal-ventral) of each injection site. Immediately following pre-surgical scanning, the MRI coordinates were transformed into stereotaxic coordinates and the stereotaxic surgeries were performed. During surgery, vital signs from the animals including body temperature, heart rate, blood pressure, rate of respiration, and expired gas CO_2 levels were recorded and monitored. Following surgery, the animal was regularly monitored until it recovered fully from anesthesia. One week after surgery, post-surgical scans were performed using T1-weighted high-resolution images scan and Fluid Attenuated Inversion Recovery (FLAIR) scan.

Hippocampal Operations: The hippocampal removal consisted of the dentate gyrus, the Cornu Ammonis fields, and the subicular complex. For neurotoxin injections into the hippocampus, the scalp was shaved and the skin was cut at the midline and two small craniotomies were performed on each side of the midline, just in front of bregma and above the targeted regions. To reduce any excessive bleeding, bone wax (Ethicon,

Inc., Somerville, NJ; 2.5g size) was applied around the edge of each craniotomy. The dura was then cut to allow the 30-gauge needle of a 10µl Hamilton syringe held in Kopf manipulators (David Kopf Instrument, Tujunga, CA) to be lowered at each site. Neurotoxic ibotenic acid (Biosearch Technologies, Novato, CA, 10 mg/ml in PBS, pH = 7.4) was infused bilaterally in 7-8 sites along the hippocampus and .6-.8µl was injected at each site at a rate of 0.2µl/30 sec for a total of 3.4-6µl. After each injection, the needles were left in place for 3 minutes to avoid retraction of the ibotenic acid along the needle track. After careful withdrawal of both needles from the brain, each needle was gently cleaned with sterile absorbent tipped applicators (Harwood Products, Guilford, ME) saturated with sterile saline (0.9% NaCl) to remove any remaining ibotenic acid or brain tissue along the length of the needle and at the beveled tip, and was then prepared for the subsequent injection. Following all injections, the incision on the dura was interruptedly sewn with absorbable sutures (5.0 Vicryl with a Taper needle; Ethicon, Somerville, NJ), followed by the galea with interrupted sutures (4.0 Dexon with a Taper needle; Ethicon, Somerville, NJ), and the skin, which was sewn continuously with absorbable sutures (4.0 Dexon with a cutting needle; Ethicon, Somerville, NJ).

Sham Operations: Sham-operations consisted of bilaterally opening of the skin, skull, and dura at approximately the same location as for the hippocampal lesions, but no injections were performed. The dura, galea and skin were then sutured.

Lesion Evaluation

The extent of damage created by bilateral ibotenic acid injections was measured using the pre- and post-surgical Magnetic Resonance Imaging (MRI) scans. Using hypersignals (produced by brain edema) seen on FLAIR images, the surface area (in

pixels) of damage to hippocampus, as well as to unintended damage to adjacent areas (amygdala, perirhinal, entorhinal and parahippocampal areas TH/TF, areas TE, TEO and V2) were estimated and plotted onto the coronal template of an intact brain.

Behavioral Task

All animals were behaviorally tested in the spatial memory tasks at three ages: at 8-months as infants, at 18-months as juveniles, and at 5-6 years as adults. Three visual paired comparison tasks (see Figure 2) were presented to the animals at each time point. For each task, 10 trials were presented.

Apparatus: The monkey was seated in a primate chair (Crist Instruments, Damascus, MD) inside a testing box 30cm from a computer monitor (Dell Ultrasharp 2407WFP-HC 24 inch widescreen LCD) in a darkened room. The images were sent to the monitor via a computer controlled by the experimenter. A video camera (Sony Digital8 TRV-140), mounted above the monitor, was positioned so that the eyes of the monkey were clearly visible and their movements could be recorded. The camera output was fed into a time/date generator connected to a VCR (JVC HR-S4800U) and into a TV monitor to allow the experimenter to monitor the animal's looking behavior during the task. The cumulative familiarization time was measured using a stopwatch. A white noise generator was used to reduce external noise.

Stimuli: Subjects saw images presented on a computer using Microsoft PowerPoint presentation. Different images were used for each trial and at each age. No stimulus was repeated within or across tasks.

Basic VPC Task: On the basic VPC task, there was a familiarization period, followed by a delay period, and two retention tests. During familiarization, the animal looked at the target stimulus for a total of 30 cumulative seconds. Following familiarization, a short delay period of 5 seconds is followed by two retention tests. During the first retention test, the animal is presented with a novel stimulus and the previously familiarized stimulus. The two stimuli stay on the screen for 5 seconds once the animal initiates looking at one of the stimuli. Following a 5-sec delay, a second retention test was given with the left/right location of the two stimuli on the screen reversed to control for any right or left looking bias. A period of 30 seconds served as the inter-trial interval. The assumption of VPC task is that memory for a familiar object can be assessed by the subjects' preference to spent longer period of time viewing the novel object. Two modified versions of the basic VPC task were used to measure memory for spatial locations, memory for object-in-place association and non-spatial object recognition memory (Kazama & Bachevalier, 2003; Bachevalier & Nemanic, 2008).

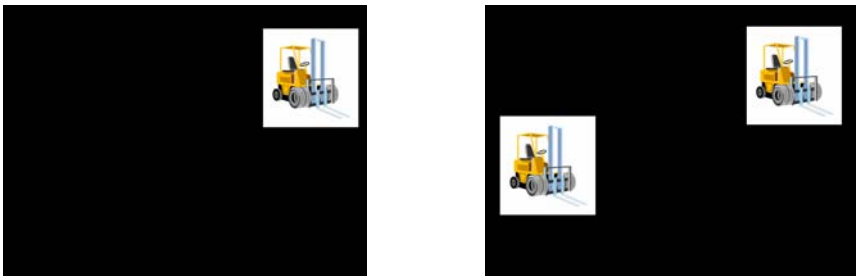
VPC-Spatial-Location: In the VPC-Spatial-Location task, preferential looking was measured by the amount of time an object in one location on the screen was viewed over the same object in another location. The familiar and novel images in this task were identical-the only difference lay in the location in which the 2 images were placed on the screen (see Figure 2A). In the familiarization phase of each trial, a single image (10 cm × 10 cm) appeared in a random location on the screen. During the retention phase, the familiar image appeared in the same location as in the familiarization phase and an identical image appeared in a different location on the screen.

Figure 2: Spatial and non-spatial visual paired comparison (VPC) tasks.

Examples of trials for the VPC-Spatial-Location (A), the VPC-Object-in-Place (B), and the VPC-Object-Control (C) tasks. Note that for the Spatial-Location task, the novel image is the same as the familiar image but is placed in a different position on the screen. For the Object-in-Place tasks the novel image differed from the familiar image only in the location of the 5 objects forming the images. In the VPC-Object-Control task, the novel image consisted of replacing three objects of the familiar image with three new objects.

VPC Tasks

Familiar Novel



A. Spatial Location

B. Object-in-Place

C. Object Control

VPC-Object-In-Place: In the VPC-Object-in-Place, preferential looking was measured by the amount of time the animal viewed one of two images, each consisting of 5 objects (3 cm × 3 cm each, and 2 cm apart) over a white rectangle background (12 cm × 20 cm) on a black screen. The familiar image consisted of a set of five objects and the novel image consisted of the same set of objects with the location of 3 of the objects rearranged (see Figure 2B). The two images appeared on the screen together during the retention test and were separated by 5 cm.

VPC-Object Control: Finally, a VPC-Object-Control task was used to ensure that any impairment in the VPC Object-in-Place could not be accounted for due to difficulty in perceiving complex visual images or due to a lack of novelty preference. In this control version, the task parameters were identical to those in the Object-in-Place task. The only difference was that the familiar image consisted of 5 objects and the novel image replaces three of the five objects with new objects (See Figure 2C). The new objects were: 1) chosen to match the color of the other objects in the image; and, 2) positioned in the same location as the replaced objects to reduce any viewing effects.

Data Analysis

Task parameters: A frame-by-frame examination of the corneal reflection of the stimuli recorded on the videotapes (see for details Pascalis and Bachevalier, 1999) was conducted to quantify four parameters: 1) the time necessary to reach cumulative 30 seconds looking at the stimulus during familiarization, 2) the total retention time defined as the actual amount of time spent fixating the stimuli during the two retention tests, 3) the percent looking time at the novel stimulus, and 4) amount of saccades per second.

Any viewing time in a trial that did not exceed one second of total looking was excluded from the analysis. Two separate observers rated the above parameters during the different ages. One observer rated all of the 8-month trials and a portion of the 18-month trials. A second observer rated the remainder of the 18-month trials and all of the adult trials. The inter-rater reliability of these observers was correlated at .95 across each task at each age.

Statistical analyses: Preliminary analyses were conducted with only the control animals to assess the normal development of spatial abilities from infancy to adulthood using individual and group means. A one-sided t-test was done to assess the novelty preference of control animals. Percent looking at the novel image was compared to the chance level (50%) for each VPC task (VPC-Spatial Location, Object-In-Place, and Object-Control) across all ages (8-months as infant, 18-months as juvenile, and 5-6 years as adult).

To assess the effects of neonatal hippocampal lesion across a developmental timeline, novelty preference of neonatal hippocampectomized animals was assessed on each behavioral task using individual means and the group mean. The group mean was compared to control animals using a Student t-test at each developmental age. More specifically, to assess the effects of early damage on later performance, comparison were made between performance of the adult animals with neonatal hippocampal lesions and adult animals with neonatal sham-operations using the Student t-test. For each group mean, percent of looking time at the novel was compared to chance performance using a one-sided t-test. Nonparametric statistics were conducted when the assumptions of the parametric tests were violated. Finally, behavioral parameters measured on each task

were correlated with the percent damage (intended or unintended) to each brain region using Pearson correlations.

To assess the effects of age on performance on the three VPC tasks, performance of monkeys with neonatal hippocampal lesions and sham-operations were compared to each other at 8-months as infants, 18-months as juveniles, and as adults at 5-6 years of age. For each task parameter, two-way repeated measures ANOVAs were conducted with Group and Age serving as main factors and repeated measures for the factor Age. Significant main effects of Age were subjected to *post-hoc* tests. Nonparametric tests were performed when the assumptions of the ANOVA were violated.

Finally, to compare the effects of neonatal versus adult hippocampal lesions on the three VPC tasks, performance of adults with neonatal hippocampal lesions that had received bilateral lesions and the sham-operated controls from the present study were compared to those of animals with bilateral hippocampal lesions done in adulthood and their sham-operated controls, using a factorial between subjects analysis of variance (ANOVA). The data on the adult animals are those reported in Bachevalier & Nemanic (2008).

These comparisons were used to indicate: 1) the normal progression of spatial memory in sham controls, 2) whether neonatal lesions to the hippocampus yielded deficit in spatial memory ability in adulthood, 3) whether any deficit in spatial memory ability was related to the extent of the damage in the hippocampus and surrounding structures, 4) whether or not neonatal hippocampal lesions yielded functional compensation as compared to the adult lesions, and 5) whether as in the adult lesions, there was a deficit in memory for object-in-space associations but not in spatial location memory.

CHAPTER 3

NORMAL DEVELOPMENT OF SPATIAL MEMORY ABILITIES

Results

Viewing Behaviors.

Viewing parameters include the overall time required to accumulate 30 seconds of looking in the familiarization phase, the total time spent looking at the two images during the two retention tests, and the number of saccades per second while viewing the two stimuli during the retention tests. Averages for these three parameters for each sham-operated animal are given in Table 1 for the three VPC tasks across the three developmental ages.

Familiarization Time: As the animals matured, the familiarization time increased in all three tasks from between 68 and 83 sec at 8-months to between 209 and 251 sec at 5-6 years (Table 1A). These differences reached significance for all three tasks [$F(2, 15) = 5.925$, $p = .013$; Kruskal-Wallis, $p = .046$; and Kruskal-Wallis, $p = .023$, for the Spatial-Location, Object-in-Place, and Object Control tasks, respectively]. Thus, in both the Spatial Location and the Object-in-Place tasks, the animals took significantly longer to familiarize as adults than as infants (Tukey, $p = .01$; Mann Whitney, $p = .025$, respectively), whereas at neither age did they differ from when they were juveniles. In the Object Control task, the animals took more time to familiarize as adults than as infants and juveniles (Mann Whitney, $p = .016$; and $p = .025$, respectively), but did not differed at the two youngest ages (infants vs. juveniles).

Total Looking Time: In contrast to the familiarization time, the total time looking at the two images during the retention tests decreased from between 3.1 and 6.3 sec at 8-months to between 2.1 and 3.0 sec at 5-6 years (Table 1B). These differences reached significance for all three tasks [$F(2, 15) = 5.266$, $p = .019$; $F(2, 15) = 7.411$, $p = .006$;

Table 1: Viewing behaviors and novelty preference in sham-operated animals

Individual scores and group means for the total time accumulated in the familiarization phase (seconds), and for the mean total looking time (seconds), mean saccades per second, and percent novelty preference in the two retention tests for the three tasks: VPC-Spatial-Location, VPC-Object-In-Place, and VPC-Object-Control in the sham-operated controls (Neo-C) as infants, juveniles, and adults.

Familiarization Time (sec)

Group/Case	Spatial-Location			Object-In-Place			Object Control		
	Infant	Juvenile	Adult	Infant	Juvenile	Adult	Infant	Juvenile	Adult
Neo-C-1	76	308	249	-	172	163	95	70	308
Neo-C-2	59	54	224	79	93	165	55	63	139
Neo-C-3	123	87	344	126	109	162	71	99	207
Neo-C-4	63	133	383	54	129	427	56	141	373
Neo-C-5	65	244	70	89	253	69	79	58	65
Neo-C-6	55	50	233	66	94	489	49	41	162
Mean	***73***	***146***	***251***	***83***	***142***	***246***	***68***	***79***	***209***

Total Looking Time (sec)

Group/Case	Spatial-Location			Object-in-Place			Object Control		
	Infant	Juvenile	Adult	Infant	Juvenile	Adult	Infant	Juvenile	Adult
Neo-C 1	2.4	1.7	1.2	4.8	1.9	1.7	6.4	2.6	1.6
Neo-C 2	3.0	1.5	2.7	3.8	2.7	3.2	7.9	4.7	3.2
Neo-C 3	3.2	1.9	2.4	5.3	2.7	2.9	4.7	2.9	4.0
Neo-C 4	3.7	2.1	1.2	5.1	3.1	1.5	7.2	2.6	2.1
Neo-C 5	2.7	2.3	3.5	4.4	2.5	4.3	6.2	6.8	4.0
Neo-C 6	3.9	2.6	1.7	5.2	5.6	2.2	5.7	5.3	3.1
Mean	***3.1***	***2.0***	***2.1***	***4.8***	***3.1***	***2.6***	***6.3***	***4.2***	***3.0***

Saccades per Second

Group/Case	Spatial-Location			Object-in-Place			Object Control		
	Infant	Juvenile	Adult	Infant	Juvenile	Adult	Infant	Juvenile	Adult
Neo-C 1	1.9	3.2	3.0	1.1	3.6	3.2	1.0	2.5	3.2
Neo-C 2	1.4	2.6	1.7	1.7	2.2	2.7	0.9	1.3	2.5
Neo-C 3	1.6	3.6	1.6	1.6	2.6	3.2	1.6	3.0	2.1
Neo-C 4	1.4	1.9	3.1	1.5	1.6	2.9	0.9	2.2	2.5
Neo-C 5	1.3	1.7	1.1	1.3	1.5	1.4	1.1	1.2	1.4
Neo-C 6	1.2	2.1	3.0	1.4	1.3	2.0	1.2	1.7	2.8
Mean	***1.5***	***2.5***	***2.2***	***1.4***	***2.1***	***2.6***	***1.1***	***2.0***	***2.4***

Percent Novelty

Group/Case	Spatial-Location			Object-in-Place			Object Control		
	Infant	Juvenile	Adult	Infant	Juvenile	Adult	Infant	Juvenile	Adult
Neo-C 1	47	62	72	46	57	63	69	52	63
Neo-C 2	59	57	66	52	50	61	71	67	66
Neo-C 3	55	57	70	48	46	56	62	67	65
Neo-C 4	58	55	52	48	59	71	73	55	74
Neo-C 5	69	83	63	52	48	52	58	69	65
Neo-C 6	42	64	73	54	55	58	71	57	62
Mean	***55***	***63***	***66***	***50***	***52***	***60***	***67***	***61***	***66***

and F (2, 15) = 9.964, p = .002, for the Spatial Location, Object-in-Place, and Object-Control tasks, respectively]. In the three tasks, the animals looked at the stimuli longer as infants than as juveniles and adults (all Tukeys, $p < .05$), but did not differ at the two oldest ages (juveniles vs. adults).

Number of Saccades: Finally, the average number of saccades per seconds increased in the retention tests for all tasks from between 1.1 and 1.5 at 8-months to between 2.2 and 2.6 at 5-6 years (Table 1C). These differences reached significance for all three tasks [Kruskal-Wallis, p = .032, F (2, 15) = 4.460, p = .03, and F (2, 15) = 8.276, p = .004, for the Spatial Location, Object-in-Place, and Object Control tasks, respectively). In the Spatial Location task, the animals made fewer saccades per second as infants than as juveniles (Mann Whitney, p = .004), but at neither age did they differ from when they were adults. In the Object-in-Place and the Object-Control tasks, the animals made significantly fewer saccades per second as infants than as adults (Tukeys, $p < .05$), but at neither age did they differ from when they were juveniles.

Novelty preference.

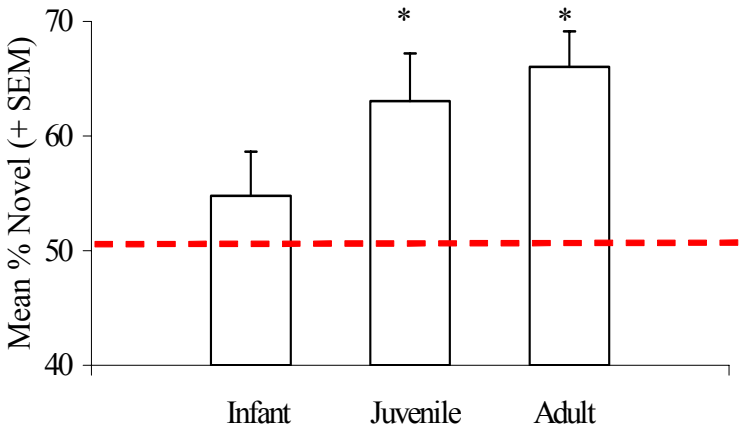
As shown in Table 1D and Figure 3, novelty preference increased with age for the Spatial Location and Object-in-Place tasks but not for the Object Control task.

VPC-Spatial-Location Task: Mean percent preference for viewing the familiar object in the new location for sham-operated controls was greater when they were juveniles and adults than when they were infants, although this difference did not reach significance (Figure 3A). However, only four of the six animals displayed preference

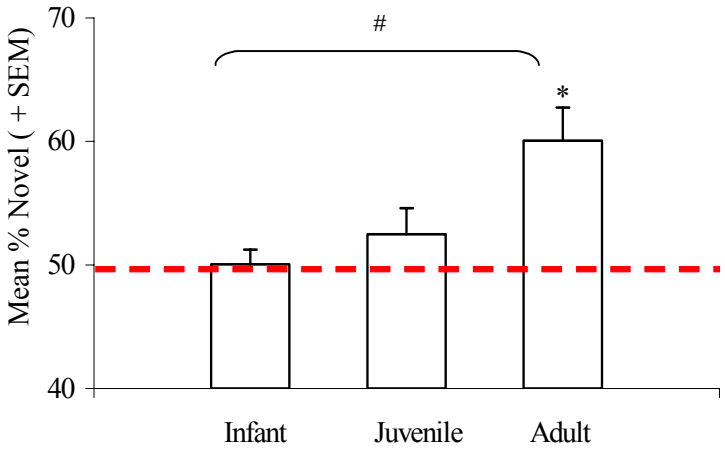
Figure 3: Novelty preference in VPC tasks.

Mean percent of time looking at the novel image (± SEM) in the VPC-Spatial-Location (A), VPC-Object-In-Place (B), and VPC-Object-Control (C) tasks for sham-operated controls (Group Neo-C) as infants, juveniles, and adults. The red horizontal dashed line represents chance performance. Asterisk defines significant difference from chance ($p < .05$) and pound represents significant difference between ages ($p < .05$).

A


Mean % Novel (+ SEM)
70
60
50
40
*
*
Infant
Juvenile
Adult

B


Mean % Novel (+ SEM)
70
60
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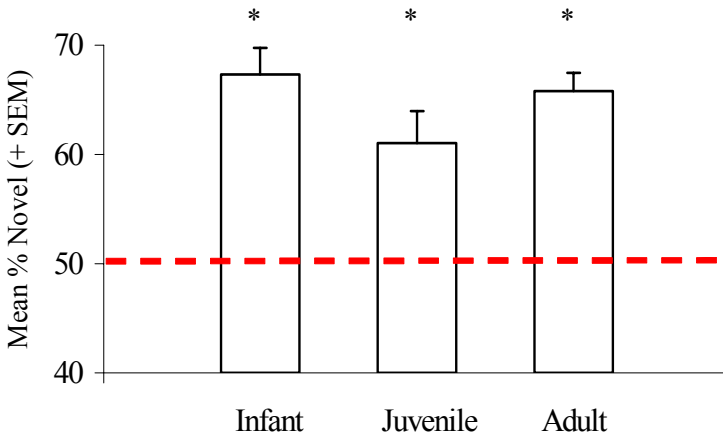
Infant
Juvenile
Adult

C


Mean % Novel (+ SEM)
70
60
50
40
*
*
*
Infant
Juvenile
Adult

scores above chance when they were infants, whereas all animals showed preference scores above chance when they were juveniles and adults, except one adult. Thus, the group mean as infants did not significantly differ from chance ($M = 54.78$, $t = 1.236$, $p > .05$), but it did when they were juveniles and adults ($M = 63.05$, $t = 3.140$, $p = .026$, and $M = 66.02$, $t = 5.133$, $p = .004$, respectively).

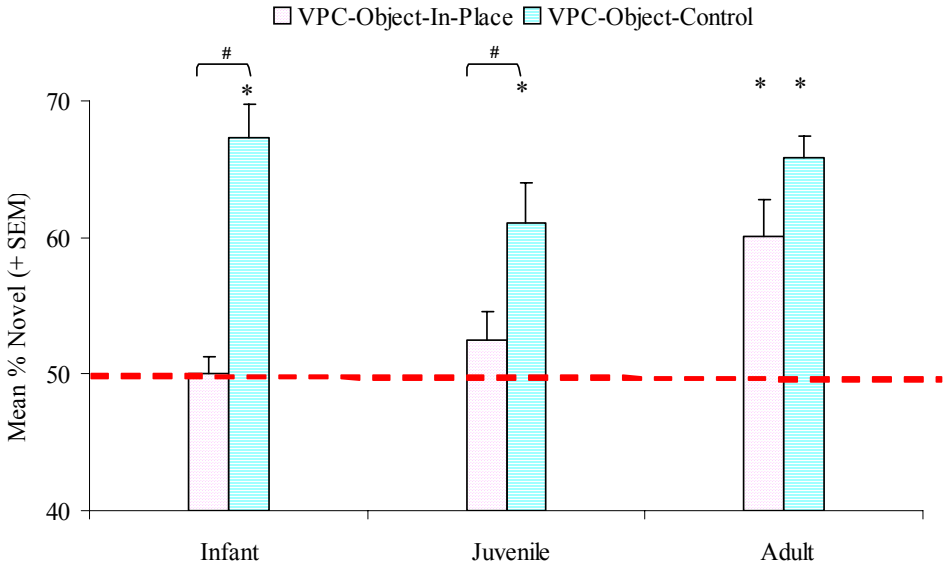
VPC-Object-In-Place Task. In the Object-in-Place task (Figure 3B), preference for novelty increased as the animals matured as revealed by a significant main effect of age [$F (2, 15) = 6.282$, $p = .01$]. Post hoc analyses revealed a significant difference between the scores obtained as infants and those obtained as adults (Tukey, $p = .01$), whereas scores obtained as juveniles fell just in between and did not differ from those of infants or adults. Thus, only as they reached adulthood, did the animals' novelty preference scores differ significantly from chance ($M = 60.07$, $t = 3.749$, $p = .013$).

VPC-Object-Control. For this control task, animals' novelty preference scores did not differ across ages and were significantly different from chance at all ages ($M = 67.32$, $t = 7.050$ $p = .001$; $M = 61.03$, $t = 3.748$, $p = .013$; $M = 65.80$, $t = 9.751$, $p = .000$ for infants, juveniles, and adults, respectively).

VPC-Object-in-Place/VPC-Object-Control Comparison. Comparisons between novelty preference in the VPC-Object-In-Place, which measures memory for object-place associations, and that of the VPC-Object-Control, which measures object recognition memory, are displayed across the three ages (infant, juvenile, and adult) in Table 1 and Figure 4.

Figure 4: Novelty preference in the VPC-Object-In-Place and VPC-Object-Control tasks.

Mean percent of time looking at the novel image in the VPC-Object-In-Place and VPC-Object-Control tasks for animals with neonatal sham operations (Group Neo-C) as infants, juveniles, and adults. The red horizontal dashed line represents chance performance. Asterisk defines significant difference from chance ($p < .05$) and pound represents significant difference between tasks ($p < .05$).



As was described earlier, the control animals looked at novelty significantly more than chance at all ages on the Object Control task, but only as they were adults for the Object-In-Place task. However, the task × group interaction failed just short of significance [$F(2, 20) = 3.370$, $p = .055$]. Nevertheless, preference for novelty was significantly greater in the Object Control task than in the Object-in-Place tasks for the infants and the juveniles [Infants: $t = -6.330$, $p = .000$; Juveniles: $t = -2.360$, $p = .04$], but not for the adults.

Discussion

The data for the sham-operated animals indicate that viewing behaviors change with maturation of the animals. This was also true for novelty preference in the two VPC tasks measuring spatial memory but not for novelty preference in object memory. Furthermore, memory for spatial locations seemed to emerge earlier than the ability to form object-place associations. These findings will be discussed in turn below.

Age Influences on Viewing Patterns

For the three tasks, sham-operated animals were faster at reaching familiarization criterion as infants than as adults. These results may indicate that with age the animals were less attentive to the stimuli or rather were able to extract visual information from the stimuli more rapidly and thus were less interested to look at the stimuli for long periods of time. This second conclusion receives support for another viewing measure, the total time looking at the two stimuli during the retention tests, which also was longer in the infants than in the juveniles and adults. In addition, the number of saccades between the two images increased with age. Thus, as infants, sham-operated monkeys appear to

spend more time viewing the stimuli and appear to make less saccades than at older ages, suggesting some important changes in visual processing abilities.

Visual System Maturation: The differences in viewing parameters observed in this investigation may suggest some modifications in visual processing abilities marked by neural changes during postnatal maturation of the visual system. As a result of the types and patterns of synaptic connections formed throughout development, neurons in the visual pathway are fine tuned to respond to specific features of a visual scene (see for review Huberman et al, 2008). In the macaque, the formation of these synapses occurs prenatally in primary visual cortex (V1) and continues postnatally, whereby synaptic density reaches its maximum by the third postnatal month (Bourgeois & Rakic, 1993). Consequently, some visual cortical functions are present early in the postnatal development of monkeys (Rakic et al, 1994). Rapid improvements in spatial vision are evident early in infancy (Maurer & Lewis, 2001). However, despite neurons in subcortical and cortical regions of the infant visual system being responsive to visual stimuli, the spatial resolution and contrast sensitivity of these cells and of cells in the retina are still immature and correspond with limited behavioral performance, as identified by preferential looking tests (see for review Blakemore, 1990; Maurer & Lewis, 2001). Due to these limitations in visual processing, infant rhesus macaques may need longer time to fixate on visual scenes in order to gather relevant information.

In addition, development of visual abilities is largely influenced by visual experience (Bourgeois, 2001). Learning and experience weaken imprecise connections in visual areas, which are ultimately eliminated, whereas connections that are more precise become strengthened and are sustained (see for review Huttenlocher & Dabholkar, 1997;

Huberman et al, 2008). Synapse elimination occurring late in childhood and into adolescence in rhesus monkeys is followed by a slow decline in synaptic density during the adult years (Huttenlocher & Dabholkar, 1997). These refinements may be related to the enhancement of spatial resolution and contrast sensitivity and have been linked to the improvement of behavioral performance on preferential looking tasks as animals mature (Blakemore, 1990). Accordingly, synaptic changes may increase efficiency in visual processing so that adult rhesus monkeys may not require prolonged looking in order to extract relevant information from the visual stimuli. Thus, one explanation for the increase in habituation times across all VPC tasks may be that adult monkeys become restless when required to fixate for extensive periods of time. In summary, experience along with a maturing visual system may result in visual abilities that assist in completing cognitive tasks and may highlight the efficiency of coding that emerges as a result of the plasticity of synaptic organizations in the visual cortex during development (Blakemore, 1990). Thus, one important question for consideration is: How might these changes in visual system maturation impact the development of spatial memory?

Age Influences on Novelty Preference

On both spatial tasks, novelty preference became stronger with age. Although novelty preference was absent in both the Spatial Location and Object-in-Place VPC tasks at the youngest age of 8 months, significant increase in novelty preference emerged at 18-months of age for the Spatial Location task, but not on the Object-in-Place task . In fact, for the Object-in-Place task, significant increase in novelty preference was present only in adulthood, suggesting that spatial abilities supporting this task emerge after 18-

months. Thus, memory for spatial location appears to emerge earlier than memory for spatial relationships among objects. One possible explanation for this protracted development of spatial memory may relate to the developmental changes in the visual processing abilities described previously. One might argue that the youngest monkeys have poorer novelty preference not because of poorer spatial abilities, but rather because of poorer visual abilities. Thus, during infancy, monkeys may not only require more time to inspect objects and extract visual information from them, but they also may be unable to distinguish minute changes between the familiarized and manipulated images regardless of whether the change be in location or in the physical attributes of the image. However, this explanation is unlikely when considering the comparisons of novelty preference between the Object-in-Place and Object Control tasks (Figure 4). Although for both tasks each trial displayed an array of five objects, hence used a similar level of visual complexity, novelty preference in the infants was clearly present in the Object Control task but not in the Object-in-Place task. These results suggest that the differences in the infants' performance on the two tasks relate to differences in cognitive processes rather than differences in visual abilities. The Object Control task is based only on a familiarity/novelty judgment (object recognition memory) since there are three new objects on the new display of each trial. By contrast, in the new display of the Object-in-Place task, all five objects are familiar so judgments cannot solely be based on memory for the familiar objects but rather on memory of the spatial relationships between the familiar objects (e.g. memory for place associations). Thus, the data suggest that memory processes based on familiarity/novelty judgment appear to be present in early infancy, whereas spatial memory processes have a more protracted development in

monkeys, with memory for spatial location emerging earlier than memory for spatial relationships.

Early and Late Developing Memory Abilities

The early developing object recognition abilities have already been described in monkeys (Bachevalier & Mishkin, 1984). Thus, using novelty preference as an index of recognition memory, Gunderson and Sackett (1984) and Bachevalier and colleagues (1993) showed that object recognition is present in the first few weeks of life in monkeys. However, development of spatial memory abilities in monkeys has only been assessed recently. The first study to test spatial memory abilities in monkeys indicated that spatial relational memory abilities, as measured with an open field memory task, are present in infant monkeys as young as 9 months of age (Lavenex and Lavenex, 2006), around the same age as the monkeys in the present study were first tested. This finding thus diverged from those reported here. The most parsimonious explanation for these dissimilar results may relate to task differences and thus to the type of spatial memory processing they measure. The current task is incidental in nature, requires no learning, and depends on a natural tendency of primates to prefer novelty, whereas the spatial task in the Lavenex and Lavenex (2006) study requires the animals to formulate a foraging strategy using spatial information from the surrounding. In this latter study, animals were placed in an open arena containing 18 identical baited cups arranged in two circular rows; an outer row contained 12 cups and an inner row contained 6 cups. Nine-month-old infant monkeys were able to locate baited cups when relational strategies were required for foraging. However, constraints in the task design of the Lavenex and Lavenex (2006)

study could have influenced their results. First, because the cups were overturned by the monkey and not replaced after the monkey made a selection, the animals were able to visually identify previously visited locations and not return to them. This makes it difficult to assess whether the selections made by the animals are the result of a spatial relational strategy or a strategy contingent upon knowledge that a specific location had already been visited. Thus, it is possible that the good performance on the task by 9-month-old infant monkeys may not truly reflect the presence of spatial association memory abilities per se. Furthermore, the protracted development of spatial memory abilities reported in the present study parallels that found in both humans and rodents. In both species, it has been shown that spatial memory abilities have a protracted development.

For example, although newborn infants demonstrate visual recognition memory (see for review de Haan et al, 2006; Pascalis et al, 1998; Pascalis & de Schonen, 1994) and even though young infants are aware of the spatial locations of objects (Newcombe et al, 1999), it is not until around age 2 that children can use visible landmarks to search for a hidden toy after a short delay (Newcombe et al, 1998; Sluzenski et al, 2004). Furthermore, preschool children are able to use spatial representation in a small environment to locate a target (Pentland et al, 2003) and showed an improvement over younger children (age 2) in their spatial ability (Foreman et al, 1983). However, young children are unable to use relational strategies in helping them locate a target (Newcombe et al, 1998; Sluzenski et al, 2004), and preschool and school-age children show poorer spatial abilities until around 7 years of age (Lehnung et al, 1998; Leplow et al, 2003; Overman et al, 1996; Pentland et al, 2003) as compared to older children and adults.

Thus, the normal maturation of spatial memory abilities in humans seems to indicate that, although children can perform some spatial tasks at approximately 5 years of age, children do not reach adult-level of proficiency until late childhood (Pentland et al, 2003; Leplow et al, 2003; Lehnung et al, 1998; Overman et al, 1996). More importantly, it appears that at different ages children may use strategies different from those used by adults to solve spatial tasks.

Likewise in rodents, adult-like proficiency of spatial memory has been demonstrated approximately three weeks after birth, around the same time as neural maturational changes in the dentate gyrus (see for review Alvarado and Bachevalier, 2000; Galea et al, 1994; Green & Stanton, 1989; Rudy & Paylor, 1988; Rudy et al, 1987). Rudy and colleagues (1987) found that rats between 21 and 23 days exhibited marked improvement in performance during training on the Morris water maze and were able to effectively use distal cues to locate a hidden platform, whereas younger rats did not. Furthermore, these authors found that older rats spent half of their search time in a quadrant of the circular maze that had previously contained a platform, whereas younger rats (under 19 days old) did not discriminate between quadrants (Rudy et al, 1987). Nevertheless, younger rats were able to successfully learn to locate a visually cued platform (Rudy & Paylor, 1988; Rudy et al, 1987), which suggests that at a younger age animals do not use spatial information provided by the environment to locate the hidden target. Interestingly, similar results were found in a different rodent species. Galea et al (1994) found that post-weaning voles were not only able to acquire the Morris water maze task faster than the younger group, but were able to retain the task while the pre-weaning voles could not. These authors observed adult proficiency of place learning in

voles by 25 days of age, which is consistent with the adult-like proficiencies evidenced at similar ages in other strains of rodents (Tonkiss et al, 1992). Using a different task, Green and Stanton (1989) found evidence of spatial memory only in older rats (21-27 days) for the more complex discrete trials of spontaneous alternation, during which successive trials followed a random instead of a regular sequence. Additional studies have indicated adult-like spatial abilities including spatial discrimination and spontaneous alternation in post-weaning rodents (Brown et al, 2005; Schenk, 1985). To sum, the developmental delay in the appearance of spatial/relational memory abilities found in monkeys are in line with those reported in humans and rodents. Our data also demonstrate a maturational distinction in the emergence of memory for locations and memory for more complex spatial relational abilities.

Neural Substrate of the Delayed Development of Spatial Memory Abilities

The importance of the hippocampus in spatial memory has been extensively demonstrated in many animal species, such as birds, rodents, monkeys and humans (Nadel, 1991; Sherry and Duff, 1996; Hampton and Shettleworth, 1996a, b; Redish, 2001; White et al, 2002; Hampton et al, 2004; Lavenex et al, 2006; Bachevalier and Nemanic, 2008; and Kessels et al, 2001 for review). More recent studies have suggested that the medial temporal cortical areas are also critical for some spatial abilities (Wiig & Bilkey, 1994 a, b; Liu & Bilkey, 2001; Steffanach et al, 2005; Buffalo et al, 2006). Furthermore, using spatial VPC tasks similar to those selected in the present study, Bachevalier and Nemanic (2008) demonstrated that memory for spatial associations was severely impaired by selective lesions of the hippocampal formation and the

parahippocampal cortex, whereas memory for spatial location was impaired only by lesions of the parahippocampal cortex. Thus, the different developmental time course in spatial ability taxed in the Spatial Location and Object-In-Place tasks may reflect distinct timelines in the functional maturation of the structures thought to mediate these spatial memory processes. Although there is very little information on the structural maturation of the parahippocampal cortex in monkeys, for the hippocampal formation evidence so far suggests a protracted development that continues until early adolescence (for review see Alvarado & Bachevalier, 2000; Lavenex et al, 2007a; Seress, 2007). Given that performance of the spatial location task seems to be mediated at least by the parahippocampal cortex, the present behavioral findings so far suggests a protracted maturation of the parahippocampal cortex, which may still reach functional maturity before the hippocampal formation. Furthermore, the results obtained in the Object-In-Place task suggest that refinements in the connections between the hippocampus, and association areas through the entorhinal cortex and parahippocampal cortex might not be fully developed until late in postnatal maturation. Nevertheless, it is also possible that delayed maturation of spatial memory abilities may be mediated by other brain structures, known to process spatial information and to have a protracted development, such as the prefrontal cortex (Goldman-Rakic, 1987; Lewis, 1997).

CHAPTER 4: EARLY HIPPOCAMPAL DAMAGE

EFFECT OF EARLY HIPPOCAMPAL DAMAGE ON SPATIAL MEMORY ABILITIES THROUGHOUT DEVELOPMENT

Results

Lesion Extent

Group Neo-H-ibo: As shown in Table 2, animals in group Neo-H-ibo were largely variable in the MRI-estimated extent of damage occurring in each hemisphere of the hippocampal formation and in surrounding medial temporal lobe areas. Bilateral hippocampal damage ranged from mild damage (33.2%) to almost complete ablation (87.4%) of the hippocampus. The mean volume reduction of the left hippocampus was 47.5%, whereas it was 66.4% for the right hippocampus. The representative case Neo-H-ibo 4 (Figure 5) illustrated the most extensive lesion of the group. Mean unintended damage indicated generally mild to slight damage to surrounding areas. Description of the hippocampal lesion in each case is given below.

Case Neo-H-ibo 1: Damage to the hippocampus was more pronounced on the right (80.9%) than on the left (54.4%). The right hippocampus was almost completely ablated, whereas the left hippocampus was almost completely spared in its posterior portion and medially along its length. Unintended damage to other areas in the medial temporal lobe surrounding the hippocampus was mild and included 21.4% and 2.7% damage to area TH/TF on the left and right, respectively. There was mild unilateral damage to the perirhinal cortex (5.4% on the left).

Case Neo-H-ibo 2: Damage to the hippocampus was more pronounced on the left (63.6%) than on the right (2.9%). Sparing was observed medially along nearly the entire length of the right hippocampus. Mild unintended damage to the amygdala (14%),

Table 2: Percent damage for members of Group Neo-H-ibo.

Intended and unintended damage to the hippocampal formation and adjacent structures in each hemisphere for all subjects in Group H-ibo. Mean- represents average damage for the group; L% - refers to percent damage in the left hemisphere; R% - refers to percent damage in the right hemisphere; X% - refers to averaged damage to both hemispheres; W% - refers to weighted average damage to both hemispheres (W% = (L% × R%)/100); ERh- entorhinal cortex; PRh- perirhinal cortex; TH and TF: cytoarchitectonic fields of the parahippocampal gyrus as defined by von Bonin and Bailey (1947). Table taken from Goursaud and Bachevalier (2007).

	Intended Damage				Unintended damage																			
	Hippocampal Formation				Amygdala				TH/TF				TE				ERh				PRh			
Subject	L%	R%	X%	W%	L%	R%	X%	W%	L%	R%	X%	W%	L%	R%	X%	W%	L%	R%	X%	W%	L%	R%	X%	W%
Neo-H-ibo 1	54.4	80.9	67.6	44.0	0.0	0.0	0.0	0.0	21.4	2.7	12.1	0.6	0.6	0.0	0.3	0.0	0.0	0.0	0.0	0.0	5.4	0.5	2.9	0.0
Neo-H-ibo 2	63.6	2.9	33.2	1.8	14.0	0.0	7.0	0.0	3.1	0.5	1.8	0.0	0.0	0.0	0.0	0.0	2.6	0.0	1.3	0.0	0.0	0.0	0.0	0.0
Neo-H-ibo 3	20.3	67.3	43.8	13.6	0.0	4.7	2.4	0.0	15.3	0.0	7.6	0.0	1.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Neo-H-ibo 4	78.5	96.3	87.4	75.6	1.7	0.0	0.8	0.0	6.1	5.5	5.8	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Neo-H-ibo 5	20.7	84.4	52.6	17.5	0.0	4.9	2.4	0.0	6.1	4.0	5.1	0.2	0.0	0.0	0.0	0.0	0.0	1.5	0.7	0.0	0.0	0.5	0.3	0.0
Mean	***47.5***	***66.4***	***56.9***	***30.5***	***3.1***	***1.9***	***2.5***	***0.0***	***10.4***	***2.5***	***6.5***	***0.2***	***0.3***	***0.0***	***0.2***	***0.0***	***0.5***	***0.3***	***0.4***	***0.0***	***1.1***	***0.2***	***0.6***	***0.0***

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entorhinal cortex (2.6%) and area TH/TF (3.1%) was observed only in the left hemisphere. No damage was observed for the perirhinal cortex or area TE.

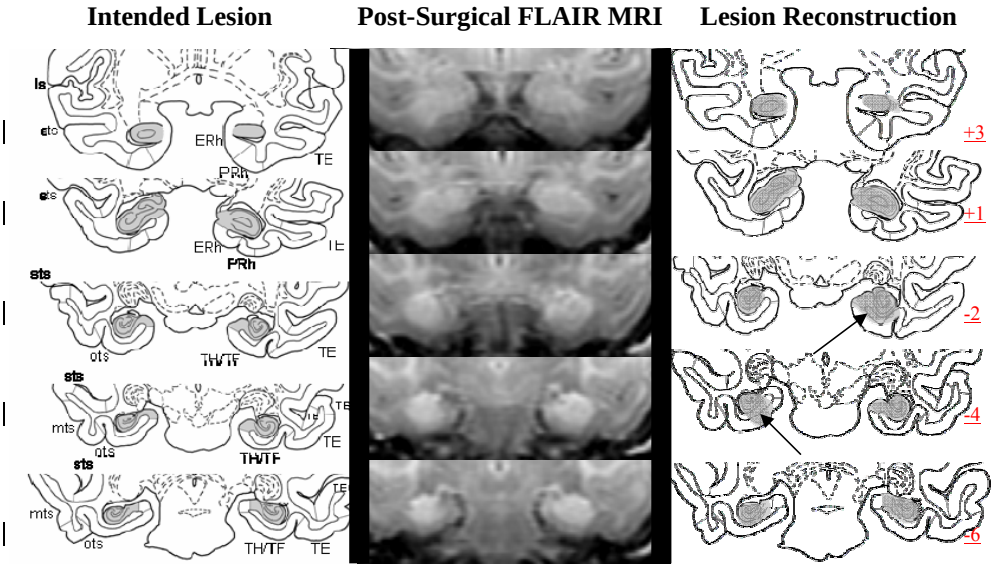
Case Neo-H-ibo 3: Volume reduction in the hippocampal was 67.3% on the right, but milder on the left (20.3%). On the left, there was sparing both at the anterior-most and posterior-most parts of the hippocampus, and sparing was observed more medially at the center of the hippocampus. Sparing to the right hippocampus was located medially in both the posterior and anterior portions of the hippocampus. Unintended unilateral damage was mild to the right amygdala (4.7%) and to area TH/TF (15.3%) on the left. Slight damage was observed to area TE on the left (1%), whereas there was no damage to either the entorhinal or perirhinal cortices.

Case Neo-H-ibo 4: Damage to the hippocampus was extensive bilaterally. However, there was more damage observed to the right hippocampus at 96.3% than to the left hippocampus at 78.5% (Figure 5). Unintended damage to the surrounding areas was mild and restricted to areas TH/TF bilaterally (6.1% and 5.5% on the left and right, respectively). Also there was slight unilateral damage to the amygdala at 1.7 % on the left. No damage was observed for area TE or the entorhinal and perirhinal areas.

Case Neo-H-ibo 5: Damage to the hippocampus was extensive on the right (84.4%) but less pronounced on the left (20.7%). Sparing on the left hippocampus was observed medially along the length and also in its anterior-most and posterior-most portions. Unintended damage was mild or slight and restricted to the right amygdala (4.9%), to the right entorhinal (1.5%) and perirhinal (0.5%) cortices, and to areas TH/TF bilaterally (6.1% and 4.0% to the left and right, respectively). There was no damage to area TE.

Figure 5: Representative Case Neo-H-ibo 4.

Coronal drawing sections through the hippocampal formation of a normal macaque brain (left column) depict intended damage (gray area). Coronal FLAIR images at corresponding levels (middle column) illustrate hypersignals (white area) resulting from edema caused by cell death. Reconstruction of the extent of hypersignals onto corresponding coronal sections of the normal monkey brain (right column). Arrows indicate areas of unintended damage. Abbreviations: ls – lateral sulcus; sts – superior temporal sulcus; ots – occipital temporal sulcus; ERh – entorhinal cortex; PRh – perirhinal cortex; TE, temporal cortical area and TH/TF – cytoarchitectonic fields of the parahippocampal gyrus as defined by von Bonin and Bailey (1947).



Viewing Behaviors

Viewing parameters (total time of the familiarization phase and total looking time and number of saccades/seconds in the retention tests) for animals with neonatal hippocampal lesions are presented in Table 3. These parameters were compared to those of control animals (see Table 1) in order to assess any changes in viewing behaviors after neonatal removal of the hippocampus.

Familiarization time: Animals with neonatal hippocampal lesions followed a similar developmental pattern as the control animals as revealed by no main effect of Group and no Group × Age interaction for familiarization time for any of the tasks. However, the two groups showed a similar increase in familiarization time across the three developmental ages as revealed by a main effect of Age [$F(2, 18) = 12.239$, $p = .000$; Huyhn-Feldt = 7.905, $p = .01$; and $F(2, 18) = 17.436$, $p = .000$; for the Spatial Location, Object-in-Place, and Object-Control tasks, respectively]. As in Group Neo-C, the total time for the familiarization phase in Group Neo-H-ibo increased in all three tasks from between 58 and 69 sec at 8-months to between 201 and 248 at 5-6 years (Table 3A). Thus, in the Spatial Location task, animals in Group Neo-H-ibo took significantly longer to familiarize as adults than as infants and as juveniles [Tukey, $p = .008$ and Tukey, $p = .049$, respectively], which did not differ from each other. In the Object-in-Place task, animals in Group Neo-H-ibo took longer to familiarize as adults than as infants and juveniles [Tukey, $p = .001$ and Tukey, $p = .044$, respectively], while juveniles did not differ from infants. In the Object-Control task, animals in Group Neo-H-ibo took longer to familiarize as adults than as infants and juveniles [Tukey, $p = .005$ and $p = .05$, respectively], which did not differ from each other.

Table 3: Viewing behaviors and novelty preference in animals with neonatal hippocampal lesions.

Individual scores and group means for the total time accumulated in the familiarization phase (seconds), the total looking time in the two retention tests (seconds), the number of saccades per second, and the percent novelty preference in the retention tests for the three tasks: VPC-Spatial-Location, VPC-Object-In-Place, and VPC-Object-Control in animals with neonatal hippocampal lesions (Neo-H-ibo) at three ages (infant, juvenile, and adult).

Familiarization Time (sec)

Subject	Spatial-Location			Object-In-Place			Object-Control		
	Infant	Juvenile	Adult	Infant	Juvenile	Adult	Infant	Juvenile	Adult
Neo-H-ibo 1	52	175	415	54	148	314	50	72	308
Neo-H-ibo 2	47	84	90	52	175	98	55	54	140
Neo-H-ibo 3	93	128	203	85	144	297	55	209	189
Neo-H-ibo 4	70	104	247	97	127	232	59	129	235
Neo-H-ibo 5	81	80	223	59	174	300	71	55	131
Mean	***69***	***114***	***236***	***69***	***154***	***248***	***58***	***104***	***201***

Total Looking Time (sec)

Subject	Spatial-Location			Object-In-Place			Object-Control		
	Infant	Juvenile	Adult	Infant	Juvenile	Adult	Infant	Juvenile	Adult
Neo-H-ibo 1	3.8	2.4	1.7	6.8	3.8	1.9	7.8	5.5	1.6
Neo-H-ibo 2	3.4	2.9	2.2	5.1	2.2	1.9	6.2	3.4	2.7
Neo-H-ibo 3	2.3	2.2	1.7	5.8	2.3	1.6	5.2	3.1	2.4
Neo-H-ibo 4	2.8	1.7	2.7	4.4	2.0	3.4	4.5	2.8	2.8
Neo-H-ibo 5	2.3	2.1	2.0	5.8	2.5	2.9	6.2	5.4	2.7
Mean	***2.9***	***2.3***	***2.1***	***5.6***	***2.6***	***2.3***	***6.0***	***4.0***	***2.4***

Saccades per Second

Subjects	Spatial-Location			Object-In-Place			Object-Control		
	Infant	Juvenile	Adult	Infant	Juvenile	Adult	Infant	Juvenile	Adult
Neo-H-ibo 1	1.0	2.1	2.1	1.1	1.7	3.7	0.9	1.5	3.7
Neo-H-ibo 2	1.3	1.1	1.6	1.5	2.3	2.8	1.1	1.8	2.9
Neo-H-ibo 3	1.5	2.0	1.7	1.1	2.6	3.5	0.9	1.8	1.9
Neo-H-ibo 4	1.7	3.1	2.1	1.6	2.5	2.4	1.5	3.3	2.2
Neo-H-ibo 5	2.0	2.7	1.2	1.3	3.8	1.9	1.4	1.8	2.2
Mean	***1.5***	***2.2***	***1.7***	***1.3***	***2.6***	***2.9***	***1.2***	***2.0***	***2.6***

Percent Novelty

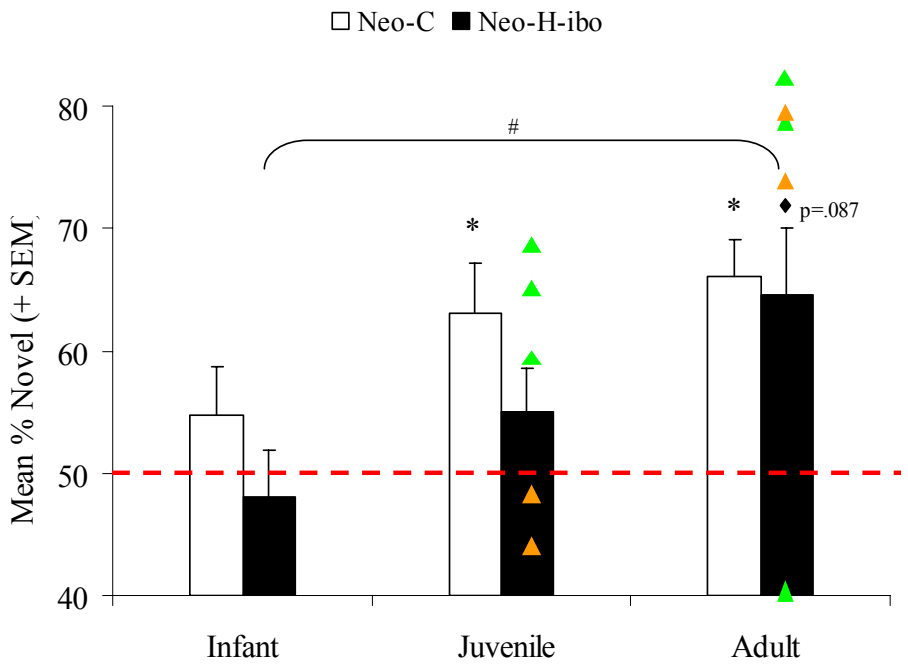
Subject	Spatial-Location			Object-In-Place			Object-Control		
	Infant	Juvenile	Adult	Infant	Juvenile	Adult	Infant	Juvenile	Adult
Neo-H-ibo 1	53	43	73	42	36	42	64	64	68
Neo-H-ibo 2	42	62	39	54	40	65	72	54	58
Neo-H-ibo 3	48	60	72	60	49	59	60	53	62
Neo-H-ibo 4	36	46	66	44	55	51	62	62	80
Neo-H-ibo 5	47	55	75	53	60	76	71	57	86
Mean	***45***	***53***	***65***	***51***	***48***	***59***	***66***	***58***	***71***

Total Looking Time: In contrast to the familiarization time, the total time looking at the two images during the retention tests decreased from between 2.9 and 6.0 sec at 8-months to between 2.1 and 2.4 sec at 5-6 years (Table 3B). These differences reached significance for all three tasks as reflected by significant main effect of Age [$F(2, 18) = 8.773$, $p = .002$; $F(2, 18) = 25.247$, $p = .000$; and $F(2, 18) = 25.439$, $p = .000$; for the Spatial Location, Object-in-Place, and Object-Control tasks, respectively]. In the Spatial Location animals in Group Neo-H-ibo looked at the two images significantly longer as infants than as adults [Tukey, $p = .045$], but looking time as infants and as adults did not differ from juveniles. In the Object-In-Place and Object-Control tasks, animals in Group Neo-H-ibo looked at the stimuli longer as infants than as juveniles and adults (Tukeys, $p < .05$), which did not differ from each other. The hippocampectomized animals did not differ from control animals in their total time looking at the stimuli in any of the three tasks, and were similar to controls at all ages as reflected by no effect of Group and no Group × Age interaction. Thus, regardless of the task, as the animals of both groups matured, they spent less time looking at the two stimuli in the retention test.

Number of Saccades: For both Groups Neo-H-ibo and Neo-C, the number of saccades per second increased as the animals matured as revealed by a significant main effect of Age [$F(2, 18) = 12.005$, $p = .008$; $F(2, 18) = 12.005$, $p = .000$; and $F(2, 18) = 16.060$, $p = .000$; for the Spatial Location, Object-in-Place, and Object-Control tasks, respectively]. However, the Group effect and the Group × Age interaction did not reach significance. Thus, as for the sham-operated controls, the average number of saccades per seconds for the hippocampectomized animals increased in all tasks from between 1.2 and 1.5 at 8-months to between 1.7 and 2.9 at 5-6 years (Table 3C).

Figure 6: Novelty preference in VPC-Spatial-Location task.

Mean percent of time looking at the novel image (± SEM) in for the sham-operated controls (Group Neo-C, white bars) and animals with neonatal hippocampal lesions (Group Neo-H-ibo, black bars) as infants, juveniles, and adults. Green triangles represent animals with mostly unilateral damage to the hippocampus (Group Neo-H-ibo$_{Unilateral}$), and orange triangles represent animals with mostly bilateral damage to the hippocampus (Group Neo-H-ibo$_{Bilateral}$). The horizontal dashed line represents chance performance. Asterisk defines significant difference from chance ($p < .05$). Diamond represents a trend for novelty preference. Star represents a trend for difference between groups ($p < .10$). Pound represents a difference between Neo-H-ibo as infants and as adults $p<.05$).



In both the Object-in-Place and the Object-Control tasks, the animals with neonatal hippocampal lesions made significantly fewer saccades per second as infants than as adults [Tukey, p = .007 and Tukey, p = .008, for the Object-in-Place and Object-Control tasks, respectively], and in the Object-in-Place task, they made fewer saccades as infants than as juveniles [Tukey, p = .022]. Juvenile animals did not differ from adults in the amount of saccades made per second in either task.

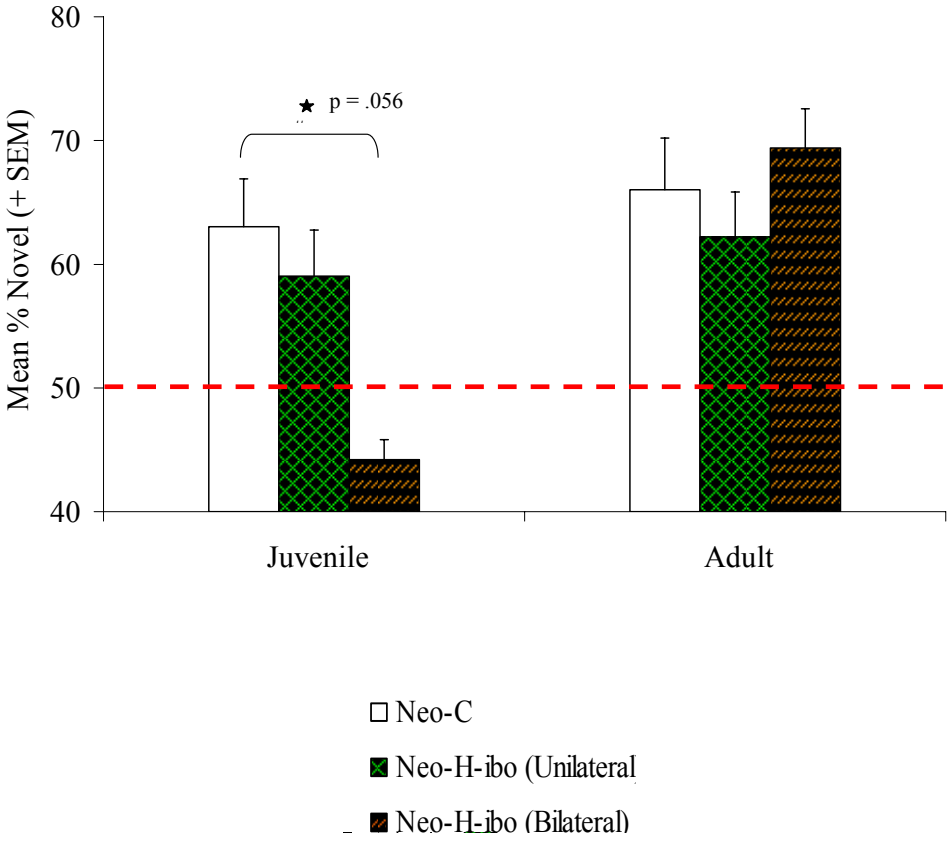
Novelty Preference

Table 3D represents the individual scores and the group means for animals with neonatal hippocampal lesions. Figures 6-11 compare mean novelty preference scores from Groups Neo-H-ibo and Neo-C for the Spatial Location, Object-in-Place and Object-Control tasks, respectively.

VPC-Spatial-Location Task: Whereas only one of the five infants with hippocampal lesions displayed preference scores above chance, three of the juveniles and all but one of the adults (Case Neo-H-ibo 2) showed preference scores above chance, however these differences failed just short of significance[Age effect: $F(2, 8) = 4.130$, $p = .059$, see Figure 6]. Comparison revealed that infants preferred novelty less than adults on this task, ($p = .030$), but the difference between the juveniles and adults did not reach significance. The group mean for infants and juveniles did not significantly differ from chance, but there was a trend for novelty preference in adults [$M = 65$, $t = 2.258$, $p = .087$]. Nevertheless, the novelty preference of the hippocampal animals did not differ from that of control animals at any of the three ages. Because we found major lesion extent differences in the animals with hippocampal lesions, we divided the animals

Figure 7: Between group differences in novelty preference in the VPC-Spatial-Location task.

Mean percent of time looking at the novel image (± SEM) for sham-operated controls (Group Neo-C, white bars) and animals with neonatal hippocampal lesions (Groups Neo-H-ibo$_{Unilateral}$ (checkered green bar) and Neo-H-ibo$_{Bilateral}$ (dashed orange bars) as juveniles and adults. The red horizontal dashed line represents chance performance. Star represents a trend for difference between groups ($p < .10$).



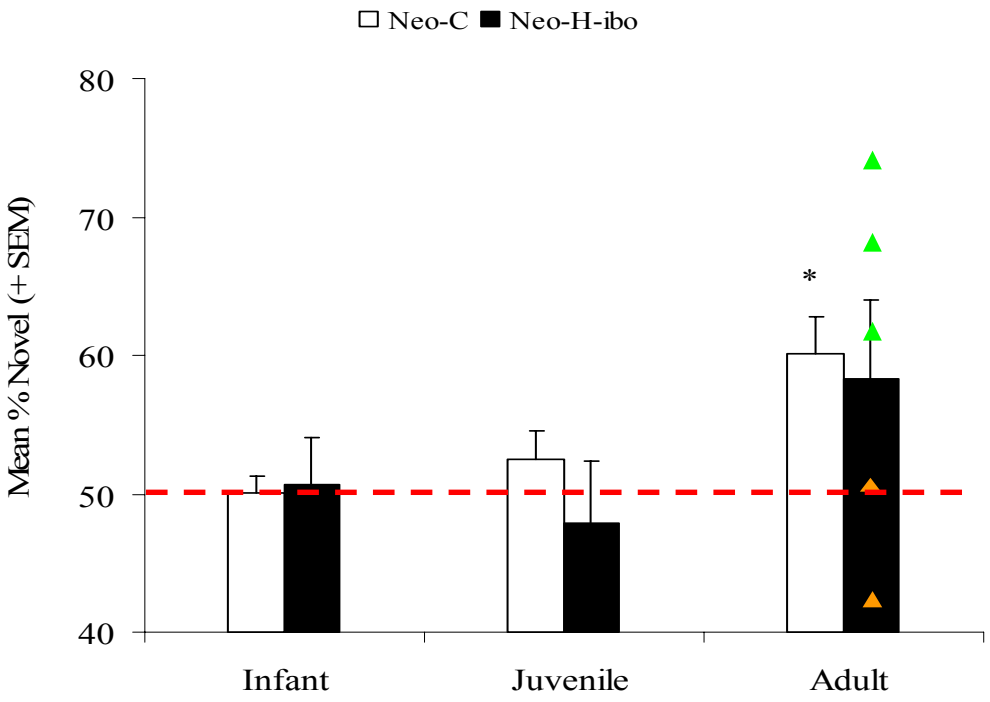
between those with mostly unilateral lesions (Cases Neo-H-ibo 2, 3, and 5 shown in green triangles on Figure 6) and those with mostly bilateral lesions (cases Neo-H-ibo 1 and 4, shown in orange triangles in Figure 6). Monkeys with unilateral hippocampal lesions showed novelty preference scores very similar to controls at all ages (all Tukeys, $p > .05$), whereas those with bilateral lesions differed from the controls as juveniles (Tukey, $p = .056$) but not as adults (Figure 7). Thus, only bilateral hippocampal lesions impacted novelty preference and only at the juvenile age.

VPC-Object-In-Place: In the Object-in-Place task (Figure 8), as compared to the sham-operated controls, preference for novelty did not increase as the hippocampectomized animals matured. Although at each age at least two of the animals were above chance (Table 3D), scores obtained at the three ages did not differ from chance [M = 49.34, 49.90 and M = 58.31, respectively]. There was a main effect of Age [$F(2, 18) = 4.722$, $p = .021$], but no main effect of Group and no Group ×Age interaction. As infants and juveniles, animals with lesions of the hippocampus performed similarly to sham-operated controls and both groups did not differ from chance. As adults, animals with neonatal hippocampal lesions did not differ from chance, whereas the sham-operated control did ($M = 60.07$, $t = 3.749$, $p = .013$). Again, the lack of group difference at the adult age may be related to the large variability in the novelty preference scores of Group Neo-H-ibo (Figure 8).

As adults, the three hippocampectomized animals that performed as well as the sham-operated controls had mostly unilateral lesions (Figure 8, green triangles), whereas the two that showed impairment had more complete bilateral lesions (Figure 8, orange triangles). Comparisons between the three groups as adults (Figure 9) revealed that the

Figure 8: **Novelty preference in the VPC-Object-In-Place task.**

Mean percent of time looking at the novel image (± SEM) for sham-operated controls (Group Neo-C, white bars) and animals with neonatal hippocampal lesions (Group Neo-H-ibo, black bars) as infants, juveniles, and adults. Green triangles represent animals with mostly unilateral damage to the hippocampus (Group Neo-H-ibo$_{Unilateral}$), and orange triangles represent animals with mostly bilateral damage to the hippocampus (Group Neo-H-ibo$_{Bilateral}$). The red horizontal dashed line represents chance performance. Asterisk defines significant difference from chance ($p < .05$).



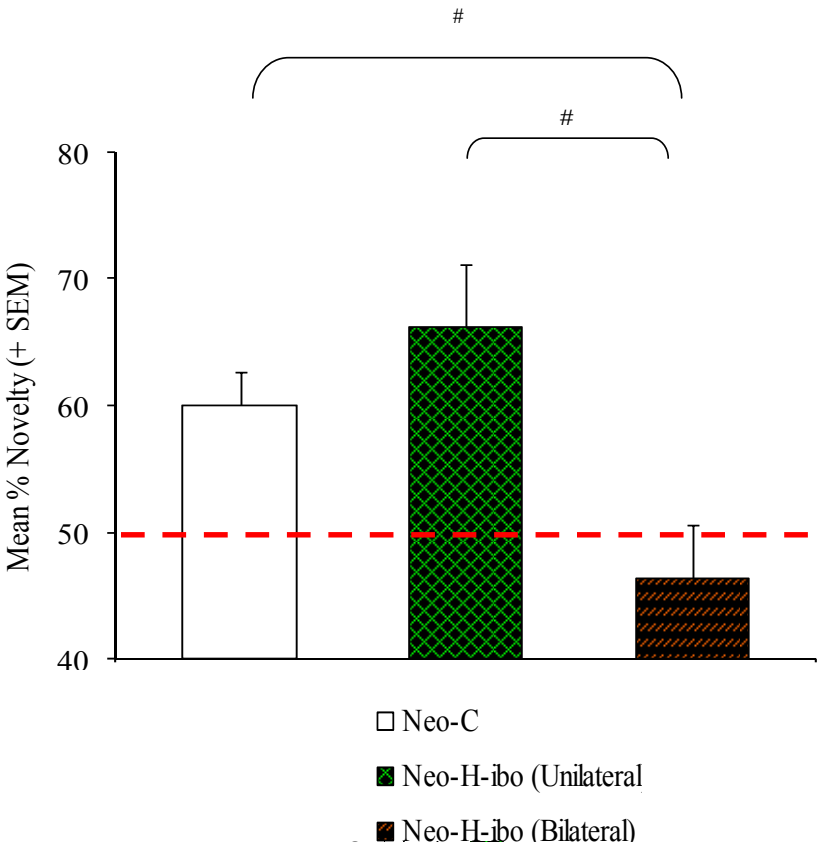
animals with bilateral lesions obtained lower novelty preference scores than both animals with unilateral lesions and controls ($p = .015$, and $p = .046$, respectively, see Figure 9).

VPC-Object-Control: For this control task, novelty preference in the hippocampectomized animals was significantly different from chance at all ages ($M = 65.24$, $t = 7.305$, $p = .001$; $M = 60.04$, $t = 3.674$, $p = .014$; $M = 71.70$, $t = 5.015$, $p = .004$; for infants, juveniles, and adults, respectively), and was similar for each age group (Figure 10). When comparing animals with unilateral and bilateral hippocampal lesions to control animals at the three ages, no differences were found between the three groups at any of the ages. Because this control task was used to ensure that any impairment in the VPC-Object-In-Place could not be due to any difficulty in visual appraisal to the complex visual display instead of difficulty in learning object-in-place associations, performance of Group Neo-H-ibo on the two tasks was also compared.

VPC-Object-in-Place/VPC-Object-Control Comparison: Novelty preference of monkeys in both Groups Neo-H-ibo$_{Unilateral}$ and Neo-H-ibo$_{Bilateral}$ was greater in the Object-Control task than in the Object-in-Place task indicated by a main effect of Task [$F (1, 8) = 14.163$, $p = .009$]. Both hippocampal groups performed similarly as revealed by no main effect of Group. However, for the animals in Group Neo-H-ibo$_{Bilateral}$ the Task × Group interaction failed short of significance [$F (1, 8) = 4.125$, $p = .089$]. Comparisons revealed that the difference between tasks was significant when the monkeys were infants and followed a trend to significance at the juvenile and adult ages [$p = .008$, $p = .059$, and $p = .088$ respectively, see Figure 11]. Furthermore, regardless of lesion size, novelty preference scores for the hippocampectomized animals were similar to controls at all

Figure 9: Between group differences in novelty preference in the VPC-Object-In-Place task.

Mean percent of time looking at the novel image (± SEM) for sham-operated controls (Group Neo-C, white bars) and animals with neonatal hippocampal lesions (Groups Neo-H-ibo$_{Unilateral}$ (checkered green bar) and Neo-H-ibo$_{Bilateral}$ (dashed orange bars) as adults. The red horizontal dashed line represents chance performance. Asterisk defines significant difference from chance ($p < .05$), pound represents significant difference between groups ($p < .05$).



and F (2, 8) = 4.793, p = .043 at the infant and adult ages, respectively]. Differences three ages in the Object-Control task, but it was not in the Object-In-Place task [F (2, 8) = 9. 833, p = .007 were observed between Groups Neo-H-ibo$_{Unilateral}$ and Neo-H-ibo$_{Bilateral}$ (Tukey, p <.05) and between the controls and Neo-H-ibo$_{Bilateral}$ (Tukey, p <.05) at these ages. This suggests normal performance for recognition of object but not for recognition of object-place associations after bilateral hippocampal lesions.

Correlation

The only significant correlations were found for the Spatial Location task and at the juvenile age only. Novelty preference correlated negatively with bilateral lesions of the hippocampus as well as bilateral unintended damage to area TH/TF (r = -.88, p < .02 and r = -.95, p <.004, respectively). These correlations indicate that at the juvenile age when Group Neo-H-ibo did not show significant novelty preference as compared to Group C, greater damage to the hippocampus and TH/TF resulted in weaker novelty preference scores. Interestingly, although we showed a dichotomy in novelty preference scores of the animals with unilateral and bilateral hippocampal lesions at the adult age, the correlation did not reach significance (see Figures 8 and 9).

Figure 10: Novelty preference in the VPC-Object-Control task.

Mean percent of time looking at the novel image (± SEM) for sham-operated controls (Group Neo-C, white bars) and animals with neonatal hippocampal lesions (Group Neo-H-ibo, black bars) as infants, juveniles, and adults. The red horizontal dashed line represents chance performance. Asterisk defines significant difference from chance ($p < .05$).

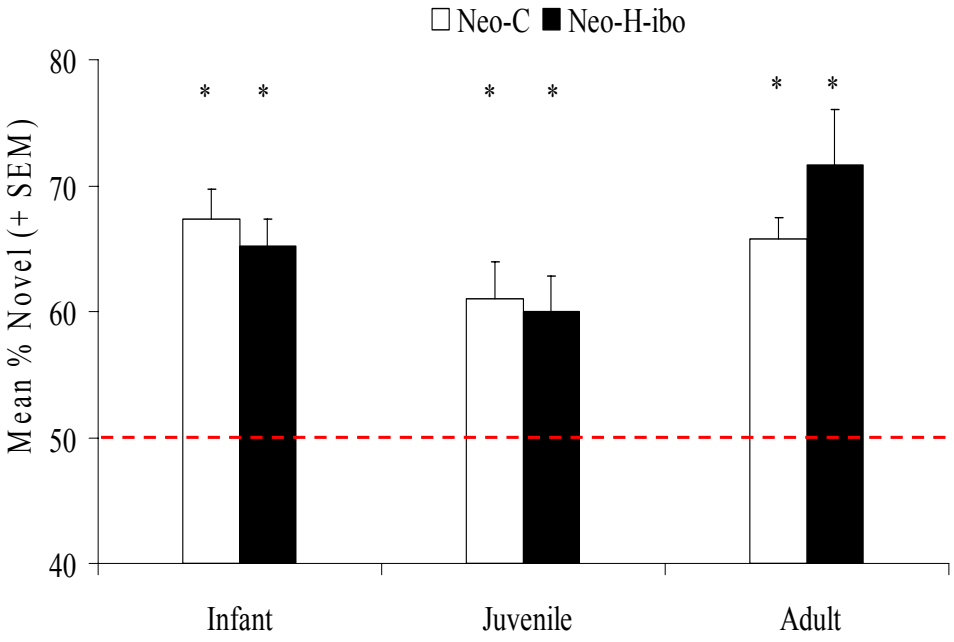
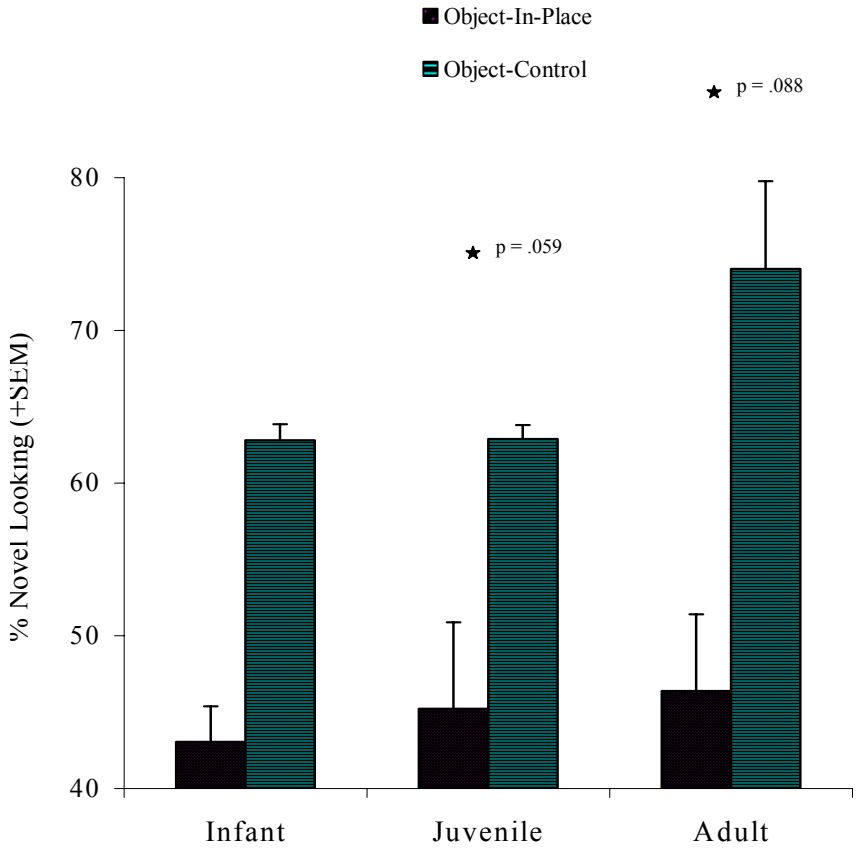


Figure 11: Novelty preference in the VPC-Object-In-Place and VPC-Object-Control tasks in animals with bilateral hippocampal lesions.

Mean percent of time looking at the novel image (± SEM) in the VPC-Object-In-Place (spotted bars) and VPC-Object-Control (Striped Bars) tasks for Group Neo-H-ibo$_{Bilateral}$ at the infant, juvenile, and adult ages. The red horizontal dashed line represents chance performance. Asterisk defines significant difference from chance ($p < .05$) and pound represents significant different between tasks ($p < .05$). Star indicates a trend for difference between tasks ($p < .10$).



Discussion

As compared to sham-operated controls, animals with neonatal lesions of the hippocampus showed normal development of visual behaviors and intact memory for object recognition, but had a protracted emergence of spatial location memory and an absence of object-place association memory. Moreover these effects were profound after bilateral but not after unilateral hippocampal lesions. Finally, the spatial memory deficits found after neonatal hippocampal lesions cannot simply be explained in terms of changes in visual perception and visual attention during maturation since the same changes were reported in the sham-operated controls (see Chapter 3). Thus, the effects of neonatal hippocampal lesions on the three VPC tasks will first be discussed in turn. Then, comparisons with the effects of neonatal hippocampal damage on spatial memory found in other species will be presented.

Neonatal Hippocampal Lesions Spared Object Recognition Memory

Object recognition memory as measured by VPC was intact in animals with neonatal hippocampal lesions at least for the short delays (5 sec) used in the present study. Thus, the sparing of object recognition memory with neonatal hippocampal lesions is consistent with the normal performance of the same monkeys found earlier with an easier version of the VPC task, in which easy discriminable color objects and short delays were used (Zeamer et al, 2006). The sparing of object recognition memory at short delays after neonatal damage to the hippocampal lesions is also in line with that obtained when the hippocampal lesions are acquired in adulthood (Zola et al, 2000; Nemanic et al, 2004; Bachevalier & Nemanic, 2008). In the first two adult studies,

monkeys with neurotoxic hippocampal lesions performed normally in a VPC task when black and white pictures of objects were used, providing that the delays between the sample presentation and retention test were less than 60 sec. In the third adult study, sparing of object recognition memory occurred when the stimuli and delays used in the VPC task were comparable to those of the present study.

Neonatal Hippocampal Lesions Transiently Impaired Memory for Spatial Location

The findings indicated that neonatal hippocampal lesions did impact memory for spatial location at an age (juvenile) when this type of memory began to emerge in the sham-operated controls. However, this effect was only transient since as adults the monkeys with neonatal hippocampal lesions showed normal novelty preference for spatial location even in animals that had more extended hippocampal lesions. Additionally, this transient deficit in memory for spatial location correlated not only with extent of damage to the hippocampus but also with extent of damage to parahippocampal areas TH/TF. The lack of impairment at the adult age is totally consistent with that of Bachevalier and Nemanic (2008) who found that selective hippocampal lesions in adult monkeys did not impact spatial location memory as measured by the VPC task. Similarly, in both the present study and the earlier one, there was no correlation between extent of damage to the hippocampus and novelty preference scores in the spatial location VPC task, suggesting that other areas, such as cortical areas TH/TF, could support this function. Indeed, the involvement of area TH/TF in spatial location memory was directly tested in the earlier adult study (Bachevalier and Nemanic, 2008) and the results showed

that direct damage to these parahippocampal areas severely impacted memory for spatial location.

Thus, in light of what we know of the medial temporal lobe structures mediating spatial location memory, the results obtained in the juvenile period are interesting and could be explained in several ways. First, the transient spatial memory deficits found in monkeys with bilateral hippocampal lesions could suggest that at this early age when memory abilities for spatial location just begin to emerge, the hippocampus may be critical to support this function, but with further maturation other structures, such as medial temporal cortical areas, could take over the function. This scenario could explain why the deficit emerges at the juvenile age but disappears when the animals reached adulthood. Therefore, the juvenile period in monkeys may be an important period in the reliance of certain spatial memory abilities on the hippocampus. However, this explanation seems unlikely given that in the Object-In-Place VPC task, which is also dependent on hippocampal functioning (Bachevalier and Nemanic, 2008), neonatal hippocampal lesions impacted novelty preference at an age when memory for spatial association is clearly present in normal animals. Alternatively, the deficit in spatial location memory in the juvenile age may not be due to neonatal damage to the hippocampus but rather to neonatal damage to areas TH/TF. Indeed, the impairment correlated not only with extent of damage to the hippocampus but also with extent of damage to areas TH/TF. Thus, a different scenario will be that the deficit found at the juvenile age could be due to mild damage to areas TH/TF, but that the deficit may not be permanent because the remaining undamaged portions of areas TH/TF could compensate for the transient loss. If this is correct, the results will suggest that memory for spatial

location is mediated by the parahippocampal areas and that during development these structures could began to function around 18-months of age.

Neonatal Hippocampal Lesions Impaired Memory for Spatial Associations

Animals with neonatal lesions to the hippocampus were impaired in the Object-In-Place task and this impairment was seen only at the adult age, when abilities to form object-space associations occurred in the sham-operated controls. This impairment cannot be associated to difficulty in perceptual decoding of complex visual stimuli since using similar stimuli the same animals with neonatal hippocampal lesions demonstrated normal novelty preference at all ages. The findings thus demonstrate that the hippocampus mediates this form of spatial memory and that its involvement in object-space association emerges after 18-months of age.

It is also interesting to note that the impairment in spatial relational memory (object-place association) confirm similar findings found on the animals described in the present study when tested in a free-foraging spatial working memory task (Glavis-Bloom et al, 2006). These two tasks are similar in that they both share a rearrangement component, i.e. monkeys' start locations changed in the food/place association task, and objects are rearranged in the object-place VPC task. Although we do not know when spatial memory abilities develop in this particular task, the data indicated impairment in discovering the location of a specific food in a large run. As for the present data, this earlier study reported that the deficit observed in the animals with neonatal hippocampal lesions was correlated with extent of damage to the hippocampal formation.

Thus, the two forms of spatial memory measured in this study indicate that memory for spatial locations and memory for object-place associations have a different developmental time course with the former emerging earlier than the latter. They also suggest that parahippocampal areas supporting memory for spatial locations appear to be maturing earlier than the hippocampal formation measuring object-place associations. This dissociation between the two types of spatial memory and of their neural substrate could in fact help explain some of the contradiction found in the monkey literature regarding the role of the hippocampus in the development of spatial memory.

Although Málková et al (1995) and Alvarado et al (2002) found clear deficits during adulthood in spatial memory in monkeys with nonselective neonatal lesions to the hippocampus on a delayed non-matching to location task, Lavenex et al (2006 and 2007b) found that spatial relational abilities as measured in an open arena task is already present by 9 months of age and are not impaired by neonatal damage of the hippocampal formation.

Málková et al (1995) investigated the long-term effect of early damage to the medial temporal lobe on learning and memory in adult monkeys on the spatial version of DNMS (Mahut & Moss, 1986). Half of the six monkeys with neonatal damage to the medial temporal lobe when tested as adults could not reach learning criterion on this task, however, with corrective training only one animal reached learning criterion while two others slightly improved performance (Málková et al, 1995). Málková et al (1995) showed that these animals exhibited a long-lasting difficulty in learning the spatial DNMS rule, which they concluded was evidence of an enduring spatial memory deficit. Another study using the same behavioral paradigm, obtained similar results. Alvarado et

al (2002) found that out of four monkeys that received neonatal hippocampal lesions, three could not learn the spatial DNMS rule within 1,000 trials. Furthermore, animals with neonatal hippocampal lesions performed consistently worse than control animals with increasing delays (Alvarado et al, 2002).

Glavis-Bloom and Bachevalier (2006) conducted an investigation in which adult monkeys (the same as used in the current study) foraged in a large scale environment for ranked foods. Animals were first given a food preference task in which their food preferences were ranked. Preferred locations consisted of foods that were ranked high for the monkey and un-preferred locations contained foods that the monkey did not usually pick during the preference task. Finally, there was one location in which no food reward was obtained. Although animals with neonatal lesions to the hippocampus learned the location of preferred foods, these animals repeatedly revisited locations that were previously foraged, which could suggest a spatial working memory deficit (Glavis-Bloom & Bachevalier, 2006).

The only investigation of spared spatial memory following neonatal damage to the hippocampus in juvenile monkeys was a recent study by Lavenex et al (2007b). These investigators suggest that monkeys with neonatal lesion show no such deficit as compared to control in spatial relational learning during a foraging task. The task used by this group was previously used in Lavenex and Lavenex (2006) and Lavenex et al (2006), and is also described in Chapter 3. Briefly, the animals were required to forage for baited cups in an open arena in which two arrays [an outer array of 12 and an inner array of 6] of 18 identical cups were placed. Lavenex et al (2007b) found that hippocampectomized animals were able to use spatial relational strategies to forage. In

addition, the authors found that the monkeys with neonatal hippocampal lesions also opened more cups, even cups that were never baited, during testing than control animals. Thus, the same constraints with this task, as mentioned earlier, apply here. The animals could use a strategy that depended not on any spatial relations but on whether a location was previously visited based on the information provided by an overturned cup. As was described above, Glavis-Bloom and Bachevalier (2006) found that when monkeys with hippocampal lesions foraged without information about previously visited sites, these animals returned frequently to already foraged locations, as indicated by a higher frequency of errors and earlier errors made during a trial. It is possible that the animals in the Lavenex et al (2007b) study would show these same effects if foraging sites were replaced after an animal visited that location.

Consequently, although Lavenex and colleagues (2007b) concluded that the lack of impairment could be attributed to reorganization of the brain due to the fact that the hippocampal lesions were done in early infancy, another likely explanation for their results could be that the spatial task used measures spatial memory functions that can be supported by other brain regions. It is worth noting that the same authors (Lavenex et al, 2006) found that impairment in the same spatial memory task after hippocampal damage in adult monkeys highly correlated with damage the parahippocampal areas TH/TF. Thus, further studies will be required to fully understand the role of the hippocampal formation and the medial temporal cortical areas in spatial memory functions.

Evidence of Protracted Development of Hippocampal-Dependent Spatial Memory in Other Species

Numerous rodent studies have assessed the effect of early damage to the hippocampus on later cellular processes and spatial memory ability in adult animals, which provide evidence that early damage to the developing hippocampus interrupts normal emergence of hippocampal-dependent memory. For instance, disruption of normal hippocampal development due to genetic factors that result in alteration of hippocampal cellular composition impairs spatial memory in mice well into adulthood (Zhao et al, 2005) and unilateral lesions to the hippocampus shortly after birth disrupted long term potentiation [LTP], activity dependent plasticity that may underlie certain forms of memory, in the contralateral hemisphere of adult rats (van Praag et al, 1998a). In another study by van Praag and colleagues (1998b), brain-derived neurotrophic factor (BDNF), a neurotrophin implicated in the survival of hippocampal cells and in the development of the hippocampus was measured following unilateral lesions to the hippocampus shortly after birth. These researchers found that in addition to spatial impairments on the Morris Water Maze, animals with neonatal lesions exhibited decreased BDNF gene expression in the contralateral hippocampus as adults (van Praag et al 1998b). In addition, a study in which methylazoxymethanol (MAM), a neurospecific agent that prevents cell division for a short period after administration and that can target select neuronal populations, was injected into pregnant females during the period in late gestation when hippocampal cells were generated in their fetuses, anatomical abnormalities was evidenced in the hippocampi of the offspring in adulthood. Also when tested on a delayed-interposed radial arm maze these MAM rats were impaired in spatial performance (Gourevitch et al, 2004). Other lesion studies found that rodents with neonatal x-ray irradiation or electrolytic hippocampal lesions tested during

adulthood took substantially longer to locate a hidden platform and displayed inferior spatial performance than adult control animals on the Morris Water Maze (Czéh et al, 2001; and, Altemus and Almli, 1997, respectively) and that greater neonatal hippocampal damage correlated with greater deficits in the spatial component of this task (Altemus and Almli, 1997). Furthermore, van Praag and colleagues (1994; 1998b) found that neonatal hippocampal lesions impaired learning to find a hidden platform in the Morris Water Maze and when tested on exploratory behaviors rats with these lesions were unresponsive to novelty (van Praag et al, 1994; 1998b).

Human studies of early damage to the hippocampus on resulting memory deficits have also shown disparate results. While Gadian et al (2000) found that on a task that measures visuo-spatial memory, patients with selective damage to the hippocampus occurring perinatally performed normally, other studies have shown deficits in spatial memory with regards to scenes and topographical information (King et al, 2004; Spiers et al, 2001; Bird et al, 2008). For instance, Bird et al (2008) using Jon, a young patient with bilateral damage to the hippocampus resulting from hypoxia-ischaemia perinatally, found that he had impaired recognition of topographical information but spared recognition of previously viewed faces relative to controls. However, they also found that when Jon was not extremely confident as to whether or not he had recently viewed particular scene his performance was poor.

Maturation of the Primate Hippocampal Formation

As described in the introduction, although almost nothing is known on the development of the medial temporal cortical areas, there is evidence now that the

hippocampal formation has a protracted development until around 2 years in the monkeys (Seress & Ribak, 1995a, b; Rakic & Nowakowski, 1981). In addition, the posterior hippocampus that has been shown to be important for spatial memory (Colombo et al, 1998; Gogtay et al, 2006) follows a distinct structural development marked by gradual increase in the volumes of most areas within this region from age 4 to age 25 in humans, but no volumetric changes was found in the total hippocampus (Gogtay et al, 2006).

With evidence from non-human and human studies, it has been speculated that the structural maturation of the hippocampus may coincide with the emergence of spatial abilities (see for examples Alvarado & Bachevalier, 2000; and, Overman et al, 1996). Thus damage to the hippocampus before full maturation may result in the interruption of hippocampal-dependent memory function at the developmental period within which this type of memory should be demonstrated (de Haan et al, 2006).

In summary, the studies in humans and non-humans suggest that there may be a critical period of postnatal development, during which hippocampal structures might be especially sensitive to environmental factors that may disrupt normal development (Lavenex et al, 2007a). While different regions of the primate hippocampal complex mature at different times (see for Review Lavenex et al, 2007a; Rakic and Nowakowski, 1981; Gogtay et al, 2006), early insult or negative environmental conditions could severely impact structural development and may be related to functional abnormalities observed throughout development (Lavenex et al, 2007a).

CHAPTER 5: TIME OF DAMAGE

COMPARING THE EFFECTS OF ADULT DAMAGE TO THE HIPPOCAMPUS WITH EARLY DAMAGE

Results

Age of Lesion Comparison

Animals with neonatal lesions of the hippocampus in the current study were compared with animals who had obtained adult lesions of the hippocampus, as previously reported in Bachevalier and Nemanic (2008), on the two spatial tasks- VPC-Spatial Location, and VPC-Object-In-Place. The monkeys with adult lesions were tested in the same behavioral paradigm at University of Texas at Houston; however, the only exception was in the VPC-Object-Control task, in which only one object in each array was replaced for the animals with adult lesions instead of the three objects replaced in each array in the current investigation.

Extent of Lesion. Table 4 (from Bachevalier & Nemanic, 2008) shows the MRI-estimated extent of damage occurring bilaterally in the hippocampal formation for animals that received adult lesion of the hippocampus (Group Adult-H-ibo). In all adult lesion cases, bilateral volume reduction was extensive and symmetric (from between 66.3% and 99.1%). The representative case Adult-H-ibo 5 (Figure 12) depicts the most extensive lesion case in this group (for all cases see Bachevalier & Nemanic, 2008). Bilateral damage in the hippocampus of animals with neonatal lesions ranged from mostly unilateral damage to almost complete bilateral ablation of the hippocampus. In addition, unintended damage to areas TH and TF in animals with adult lesions was mild to moderate, whereas animals with neonatal lesions had minimal damage to these areas. Thus, the hippocampal lesions in the adult cases (Bachevalier and Nemanic, 2008) were more extensive and bilateral than the neonatal hippocampal lesions (Chapter 4-Table 2).

Table 4: Intended damage to the hippocampal formation and unintended damage to adjacent areas.

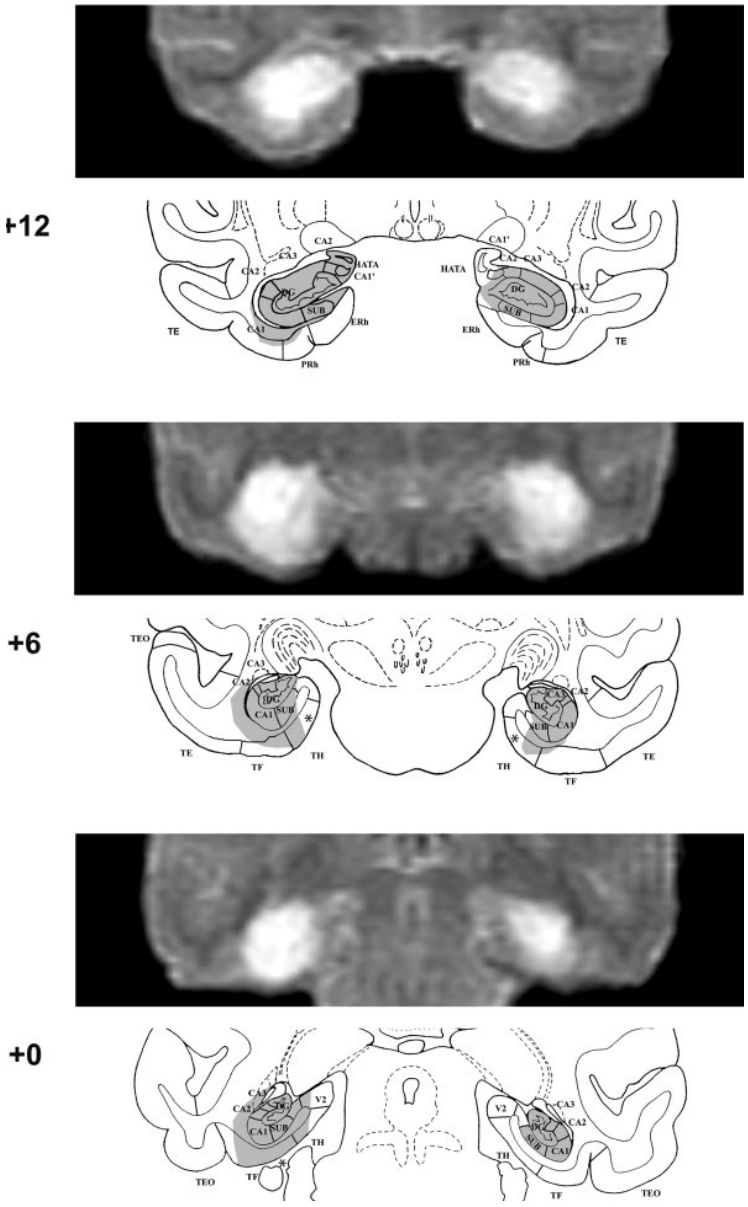
Mean average damage for the group; L% - refers to the percent damage in the left hemisphere; R% - refers to the percent damage in the right hemisphere; X% - refers to the average damage to both hemispheres; W% - refers to the weighted average damage to both hemispheres (W% = (L% × R%)/100). TH and TF: cytoarchitectonic fields of the parahippocampal gyrus as defined by von Bonin and Bailey (1947). Data are from Bachevalier and Nemanic (2008, see Table 1).

	Intended Damage				Unintended damage							
	Hippocampal Formation				TH				TF			
Subject	L%	R%	X%	W%	L%	R%	X%	W%	L%	R%	X%	W%
Adult-H-ibo 1	75.6	97.9	87.2	74.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adult-H-ibo 2	75.8	81.3	78.5	61.6	53.1	20.1	36.6	10.7	60.3	27.6	43.9	16.6
Adult-H-ibo 3	67.6	74.1	70.9	50.1	26.7	15.3	21.0	4.1	30.0	44.0	37.0	13.2
Adult-H-ibo 4	56.4	76.3	66.3	43.0	13.6	27.8	20.7	3.8	18.5	19.4	18.9	3.6
Adult-H-ibo 5	98.8	99.3	99.1	98.1	15.2	15.9	15.6	2.4	38.8	8.5	23.7	3.3
Adult-H-ibo 6	88.9	94.9	91.9	84.3	29.6	45.6	37.6	13.5	21.2	17.2	19.2	3.6
Mean	***77.3***	***87.3***	***82.3***	***68.7***	***23.0***	***20.8***	***21.9***	***5.8***	***28.1***	***19.5***	***23.8***	***6.7***

Figure 12: Extent of Lesion.

FLAIR coronal MRI (top) of representative case Adult-H-ibo (top) matched with drawing sections of a normal monkey brain (bottom) for three A-P levels through the hippocampal formation. Hypersignals representing edema cause by cell death in the MR images were reconstructed unto the corresponding drawing sections (gray area) of the normal monkey brain. Asterisk in levels +6 and +0 points to unintended damage mostly in areas TH/TF bilaterally. Abbreviations: CA1, CA2, and CA3, Cornu Ammonis Fields of the hippocampus, DG: dentate gyrus, ERh: entorhinal area 28, HATA: amygdala-hippocampus transition area, PRh: perirhinal areas 35 and 36, SUB: subicular complex, TE, TEO, TH, and TF: cytoarchitectonic fields described by von Bonin and Bailey (1947), and V2: visual extrastriate cortical area (Figure 2 in Bachevalier and Nemanic, 2008).

Adult-H-ibo 5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Novelty Preference

There were apparent differences between the animals with mostly unilateral lesions (Cases Neo-H-ibo 2, Neo-H-ibo 3, and Neo-H-ibo 5) and animals with extensive bilateral lesions to the hippocampus (Cases Neo-H-ibo 1 and Neo-H-ibo 4). Thus, to compare the animals with early lesions to those with late lesions, we used the data from animals with the lesions closest to those in the Bachevalier and Nemanic (2008) study. Since the lesions in the Bachevalier and Nemanic (2008) were bilateral and extensive, the three cases with mostly unilateral lesions to the hippocampus were excluded in this current analysis.

Spatial-Location task: As with animals that received adult lesions of the hippocampus, animals that received neonatal lesions when tested as adults preferred a novel location of a familiar object significantly more than chance (Figure 13A). None of the groups were significantly different from each other. There was no effect of group [F (1, 16) = .899, $p > .05$] or of time of lesion [F (1, 16) = .132, $p > .05$]. According to Bachevalier and Nemanic (2008) both the adult lesion group and the adult control group showed novelty preference [$t = 3.70$, $p < .0001$ and $t = 1.54$, $p < .0001$, respectively]. The previous results are thus similar to those obtained in the current investigation. The animals in Group Neo-H-ibo$_{\text{Bilateral}}$ showed a trend for novelty [$t = 6.123$, $p = .051$] while the neonatal control group also showed novelty preference for the object in the new location [$t = 5.133$, $p < .05$].

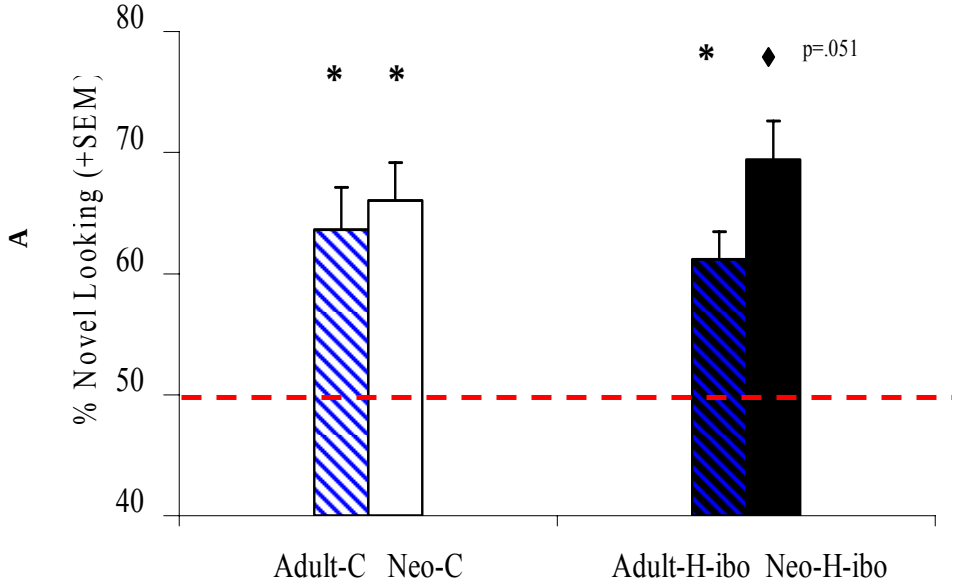
Object-In-Place task: As shown in Figure 13B, both animals with neonatal (M = 46.39) and adult lesions (M = 51.30) of the hippocampus were impaired on the Object-In-Place task, whereas control animals were unimpaired (ps < .05). There was a main effect

of group [F (1, 16) = 2.874, p = .002] but no main effect of time of lesion [F (1, 16) = .595, p > .05]. Thus, regardless of when the lesion occurred animals with hippocampal damage showed no preference for the novel rearrangement as compared to operated controls.

Object-Control task: Although the Object-Control tasks were different in the amount of objects replaced, animals in the neonatal hippocampal group was not different from controls for novelty preference (t = -2.035, p >.05) and the animals in the adult group also performed similar to controls on this task (see Bachevalier & Nemanic, 2008) even though the difficulty of the task was greater in Bachevalier and Nemanic (2008) than in the current study.

Figure 13: Novelty preference in VPC-Spatial-Location (A) and VPC-Object in Place (B) tasks.

Mean percent of time looking at the novel image for animals with adult and neonatal lesions. The red horizontal dashed line represents chance performance. Asterisk defines significant difference from chance ($p < .05$), diamond represents a trend for novelty preference, and pound represent significant difference from the age matched lesion group. Abbreviations: Groups Neo-C and Adult-C, animals receiving sham operations as infants, and adults, respectively; and Groups Neo-H-ibo and Adult-H-ibo, animals receiving neonatal neurotoxic lesions of the hippocampus during infancy and during adulthood, respectively.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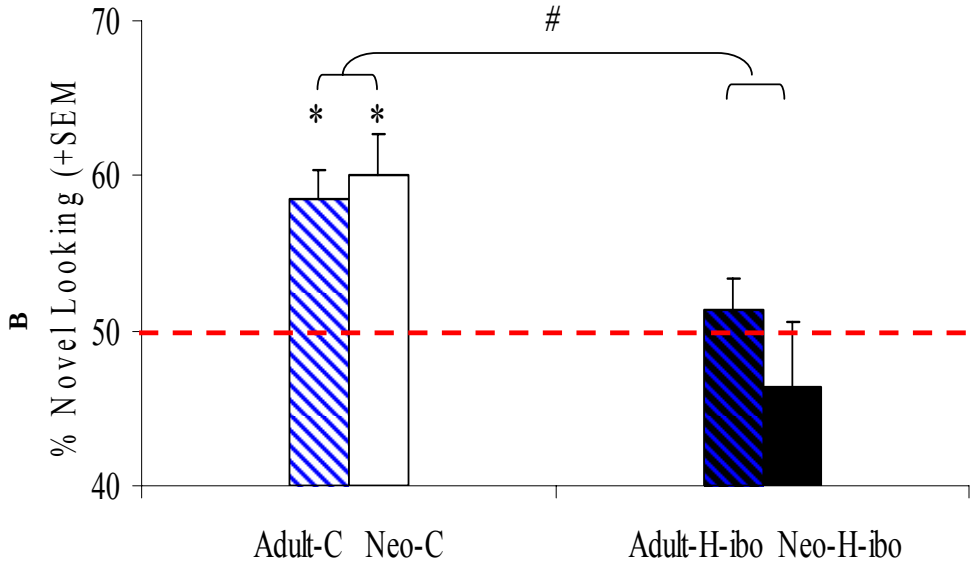
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Discussion

The goal of the current chapter was to assess whether the age at lesion had any effect on spatial memory performance. The current findings show no differences between the lesion groups in impairment of memory during adulthood. Both lesion groups show intact object recognition memory and spatial location memory, however, object-place association memory was disrupted in both groups. This leads us to conclude that the impairments seen in subjects with selective neonatal lesions to the hippocampus are pervasive throughout development and mimics selective adult lesions to the hippocampus.

As discussed in Chapter 4, several studies have suggested that reorganization of the brain after early hippocampal damage is sufficient to support associative and spatial memory (Braun et al, 2008; Lavenex et al, 2007b). However, numerous studies in several different species including the current one have suggested that early hippocampal damage is pervasive resulting in a life-long impairment in memory subserved by the hippocampus (Altemus & Almli, 1997; Alvarado et al, 2002; Bachevalier & Vargha-Khadem, 2005; Czéh et al, 2001; Gourevitch et al, 2004; van Praag et al, 1994; 1998b; De Haan et al, 2006; Málková et al, 1995; Vargha-Khadem et al, 1997; Zhao et al, 2004). De Haan and colleagues (2006) concluded that following hippocampal damage, memory systems could indeed reorganize atypically; however, they also stipulate that since in infant memory systems are more widely distributed, early damage may prevent normal more focal neural systems from developing. In addition, little developmental plasticity for certain types of memory (i.e. episodic memory) is apparent following hippocampal damage (De Haan et al, 2006).

Comparison of Age at Injury on Memory Effects

Studies in different species indicated dissimilar results, reporting either the presence or absence of spatial memory impairments, in adult animals after hippocampal injury during infancy and in adulthood. Although several studies in rodents (van Praag et al, 1994; 1998b) and primates have shown spatial memory deficits following both adult and neonatal lesions (Kessels et al, 2001; Kumaran et al, 2007; Piggott & Milner, 1993; Smith & Milner, 1981; 1989), others reported sparing of spatial memory abilities after early hippocampal lesions (Gadian et al, 2000; Lavenex et al, 2007b) and adult hippocampal lesions (Bohbot et al, 2002). Yet, only a few studies in primates have compared the age at injury when analyzing the effects of hippocampal lesions on spatial memory deficits.

One interesting study (Braun et al, 2008) assessed two groups of human patients with hippocampal resections to correct epilepsy [one group with recent onset of epilepsy (1 - 3 years) due to hippocampal tumors and one group with longstanding epilepsy (10 - 24 years) due to hippocampal sclerosis] that were tested on memory for color, location, and color-location association in a computerized task that required these individuals, following a delay, to make a match/ non-match distinction as to whether: (1) a square of a particular color was previously seen in an array of 4-6 multicolored squares [color task], (2) a square in an array of like-colored squares was previously in a particular location [location task], or (3) a colored square in an array of multi-colored square was previously in particular location [color-location task].

These investigators found that both groups were impaired relative to control subjects at long and short delays for all tasks, in the color-location task in particular,

although the tumor patients with recent epilepsy onset and smaller hippocampal lesions showed a delay-dependent drop in performance that was not seen in the sclerosis patients, who had larger hippocampal lesions. They concluded that in patients with long-standing damage to the hippocampus [they argued that it was possible for sclerosis patients to have an early onset and a latency period of many years] reorganization had to occur in order to support some delay-dependent sparing in associative memory functions, whereas no reorganization occurred in patients with recent onset of seizures in adulthood following hippocampal tumors. Even though these researchers claimed that the hippocampal sclerosis is a progressive disorder and that the epilepsy associated with it required profound damage to manifest, since only two patients with hippocampal sclerosis began having epilepsy as children (at 4 and 8 years), the findings of this particular investigation cannot lend any information to the time at which damage to the hippocampus first occurred in these patients.

Lavenex et al (2007b) compared the results of the animals with neonatal lesions tested as juveniles to animals with adult lesions to the hippocampus and found that animals with late lesions were significantly different from those with early lesions. They found that while adult lesions impaired spatial relational learning, neonatal lesions had little effect on this type of memory. Thus, they concluded that reorganization of the hippocampal memory system had to account for the sparing in spatial relational learning. However, in this study, monkeys with hippocampal lesions had also extensive damage to areas TH/TF.

Thus, de Haan et al (1997) suggest that although reorganization of the brain can occur following early damage to the medial temporal lobe, namely to the hippocampus,

the evidence suggest that the pervasive impairments in spatial memory and some other hippocampal-dependent memories cannot be overcome by compensatory neural structures or systems.

CHAPTER 6: GENERAL DISCUSSION AND FUTURE DIRECTIONS

General Discussion

This current investigation had several aims: (1) to track the developmental trajectory of spatial memory processes in normally developing macaques, (2) to evaluate the long-term effects of damage to the hippocampus occurring within a week after birth in developing monkeys during [a] infancy, [b] adolescence, and [c] adulthood, and (3) to compare the effects of early versus late damage to the hippocampus monkeys.

We clearly demonstrated a protracted development of spatial memory. However, while memory for spatial location appears during the juvenile period in the monkey, it is not until after the juvenile period, that normal monkeys are capable of memory for object-place associations. In addition, monkeys with early damage to the hippocampus were impaired as juveniles on memory for spatial location but not when tested as adults. This pattern of results suggests a transient impairment of spatial location memory. However, object-place associations remained impaired into adulthood, suggesting that damage to the hippocampus results in pervasive deficits of spatial relational memory. These data are supported by several other studies (de Haan et al, 2006; Vargha-Khadem et al, 1997; Bachevalier & Vargha-Khadem, 2005). Furthermore, we found that the long-term effects of neonatal hippocampal lesions mimic those of late damage to the hippocampus on both object-place associations and spatial location memory (Bachevalier & Nemanic, 2008).

Developmental Timelines. Developmental timelines are important in addressing both the structural maturation of the brain and its functional correlates. Of interest to Bauer (2006) is "further development of conceptual links between observed age-related changes in the basic processes of encoding, consolidation and storage, and retrieval, and

the developments in the neural substrates" that underlie these processes. We see here that the developmental timeline of hippocampal development show a delayed maturation of this structure. Thus, it is important to link the development of certain spatial memory processes to this delayed maturation of the hippocampus in normally developing animals.

Future Directions

Within our first aim, we were limited by the time points at which the monkeys were tested. At 8-months, 18-month, and 5-6 years of age, these animals were tested on the VPC task to assess the development of spatial memory in normal monkeys. However, due to the long period between testing as juveniles (18-months) and as adults (5-6 years) it becomes difficult to assess the exact time period within which spatial memory abilities emerge, namely object-place association memory. In order to obtain a more accurate picture of the development of memory for object-place associations, we plan to test normally maturing monkeys at 2 years of age and, if necessary, once again before 5-6 years of age. Repeated testing of monkeys with functioning hippocampi will give us some insight into periods in development during which spatial memory abilities appear.

As with our first aim, testing animals with damage to the hippocampus between 18-months and 5-6 years is imperative. We see in monkeys with hippocampal lesions that memory for spatial locations are impaired through adolescence. It would be helpful to assess at what point spatial location memory emerges in these monkeys to get some insight into whether there are other pathways that can support this type of memory in the absence of a functioning hippocampus. In addition, we may see evidence to indicate our

claim that memory for spatial location might be dependent on the hippocampus early on in development and as the animals mature and more focal memory processes are established, this form of memory might be supported by a structure other than the hippocampus.

Our third aim was to measure whether there were any differences between hippocampal damage occurring in infancy and damage occurring in adulthood. We found no differences in performance of animals with early or late lesions. Since we were unable to evaluate the strategies that these animals could have been using to solve our spatial task, it would be interesting to see whether monkeys with late lesions and monkeys with neonatal lesions use the same strategies to solve spatial problems by assessing their performance on open arena tasks, similar to the ones used in the Glavis-Bloom and Bachevalier (2006) and Lavenex et al (2006; 2007b) studies.

Lastly, it would be interesting to see whether human infants, school-aged children and adolescents, and adults display this same form of developmental trajectory of spatial memory abilities in a paradigm similar to the current VPC setup that is incidental in nature. In addition, being able to track the looking patterns using an eye-tracking device would give a more accurate portrayal of where within the images individuals are looking to assess whether humans across development encode spatial information similarly.

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