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MEDIAL TEMPORAL LOBE STRUCTURES AND THE DEVELOPMENT OF RECOGNITION MEMORY

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By

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M.S., University of Texas – Houston, 2004

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Abstract

MEDIAL TEMPORAL LOBE STRUCTURES AND THE DEVELOPMENT OF RECOGNITION MEMORY

By Alyson Zeamer

Object recognition memory as assessed by the visual paired comparison task (VPC) is known to emerge early in infancy in both human and non-human primates. However, neither the normal maturation of these processes, nor the developmental role of the medial temporal lobe structures thought to be involved in this form of incidental memory have been systematically examined through longitudinal studies. Therefore, this study longitudinally followed the normal maturation (Group Neo-C) of recognition memory processes as well as measured the magnitude of object recognition memory deficits seen after selective neonatal hippocampal (Group Neo-H) or perirhinal cortex (Group Neo-PRh) damage in monkeys (*Macaca mulatta*). The data showed that (1) incidental recognition emerges very early in life and, at this early age, is mediated by PRh rather than H; (2) this memory process, however, proceeds through major maturational changes after the first year of life, presumably as H becomes functional and begins competitive functional interactions with PRh; (3) equally interesting was that, although Neo-PRh lesions impacted item-specific recognition memory at all ages tested so far (1.5-18 months), this impairment was less in magnitude than that reported after PRh lesions in adulthood, suggesting that the neural substrate mediating incidental recognition memory in early infancy is more widespread than that of the adult. However, this proposal warrants further investigation since it is uncertain as yet whether performance of animals

with Neo-PRh damage will worsen with age to reach the magnitude seen with adult PRh lesions or whether developmental plasticity will further ameliorate their deficit. Finally, the data obtained in these developmental studies provide further support to the idea that the recognition processes mediated by the perirhinal cortex are different from those supported by the hippocampal formation.

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The last two decades have provided ample evidence that declarative memory processes require not only the hippocampus, but also the adjacent cortical areas. However, very little is known about the development of these memory processes and the neural substrates that support them. Therefore, this thesis is an attempt to provide new data on how the hippocampus and the perirhinal cortex may support recognition memory across development in nonhuman primates. A brief introduction on how the medial temporal lobe became the focus of studies on memory processing will be given, followed by a description of how its anatomical organization fits with this idea. A review of the literature on the contribution of two specific structures, the perirhinal cortex and hippocampus, in recognition memory processes will then be given. Finally, the little knowledge on the anatomical development of these two structures will be summarized together with their contribution to the development of recognition memory processes.

Introduction

In 1957, Scoville and Milner provided a landmark description of the well-known amnesic patient H.M. who, following a bilateral temporal lobectomy, exhibited a profound impairment in retaining and recalling virtually any kind of new information (anterograde amnesia), such as recalling short stories, word lists, pictures and a wide range of other material (Corkin, Amaral, Gonzalez, Johnson, & Hyman, 1997; Scoville & Milner, 1957). A later MRI analysis of H.M.'s brain revealed that his lobectomy included the anterior half of the hippocampal formation, the amygdala, the entorhinal cortex and parts of the perirhinal cortex and area TH/TF (Corkin et al., 1997). Because of the early description of H.M.'s memory deficits, as well as those of several other patients with

more restricted damage, the medial temporal lobe, and most importantly the hippocampus, is now thought to be involved in declarative memory.

Declarative memory is defined as memory that can be deliberately brought into consciousness, such as recollecting what you did on your last birthday, and is considered a form of explicit memory, whereas nondeclarative, or procedural memory which is not dependent on the medial temporal lobe is considered implicit (Nelson, 1995). Tulving and Markovitch (1998) further divided declarative memory processes into episodic and semantic memory, where episodic memory refers to memory for events or episodes personally experienced in your past, or autobiographical memories, and semantic memory refers to every day facts, such as "Austin is the capitol of Texas". One prevailing theory argues that the hippocampus mediates episodic memory processes, whereas the surrounding cortex mediates semantic memory processes (Brown & Aggleton, 2001; Mishkin, Suzuki, Gadian, & Vargha-Khadem, 1997; Mishkin, Vargha-Khadem, & Gadian, 1998; Tulving and Markowitsch, 1998; see Squire, Stark, & Clark, 2004 which suggests that both are dependent on the hippocampus), suggesting that declarative memory emerges from cooperative interactions among relatively specialized medial temporal lobe components. Evidence to support this proposal emerged from the anatomical organization of this brain region as well as recent advances on the role of the medial temporal lobe structures in recognition memory process.

Anatomy of the Medial Temporal Lobe

The medial temporal lobe consists of a set of structures including the hippocampal formation (CA Fields, dentate gyrus, subiculum, fimbria and fornix), amygdala,

entorhinal cortex (ERh), perirhinal cortex (PRh) and areas TH/TF (Figure 1). Although highly-processed sensory inputs reach the medial temporal cortex, these inputs seem to be loosely segregated into two different pathways (Felleman & Van Essen, 1991; Ungerleider & Mishkin, 1982; Desimone & Ungerleider, 1989; Boussaoud, Ungerleider, & Desimone, 1990). Inputs carrying perceptual properties (visual, auditory, somatosensory) of objects seem to predominantly terminate in the PRh. By contrast, inputs carrying context information as well as spatial and motion attributes of objects in space seem to predominantly terminate in areas TH/TF of the parahippocampal gyrus (ParaH). Although this dissociation of afferent inputs into the medial temporal cortex is not absolute, it seems to proceed further into the next relay station, i.e. the ERh, which receives approximately two-thirds of its projections from the PRh and ParaH (Insausti, Amaral, & Cowan, 1987) and is the largest direct projection to the hippocampus (HP). Thus, the two streams of processing remain largely segregated as the PRh projects primarily to the rostral two-thirds of ERh, whereas the ParaH projects mainly to the caudal two-thirds of ERh (Suzuki and Amaral, 1994b).

The large number of projections from the ERh to the hippocampus forms the perforant pathway, which synapses onto granule cells of the dentate gyrus (DG) (Suzuki & Amaral, 1994a, 1994b). The granule cell axons, called mossy fibers, then project to the pyramidal cells of CA3 and synapse with cells in the dentate hilar region. The CA3 pyramidal cell axons, called Schaffer collaterals, divide to project both to the pyramidal cells of CA1 and to other brain structures (septum, mamillary bodies, prefrontal cortex) via the fimbria and fornix. The major output of CA1 is the subiculum, which sends projections back to the PRh and ParaH (Lavenex & Amaral, 2000). In addition to inputs

from the perforant path, CA1 also receives direct projections from the ERh, PRh and ParaH, and much smaller projections from the cingulate, parietal and orbital prefrontal cortex, dorsal bank of the superior temporal sulcus and the insula (Suzuki & Amaral, 1994a; 1994b; Wellman & Rockland, 1997). The question that remains to be answered is how these structures participate and cooperate in recognition memory processes.

Recognition Memory

Recognition memory is defined as the ability to determine whether or not a stimulus has been previously encountered. It has been further divided into familiarity judgment, which is the subject's knowledge (or gist) that an object or event has been previously encountered, but without the ability to remember the episode itself, and recollection, which is the ability to remember all information surrounding a previous encounter. Although it is widely accepted that the medial temporal lobe cortical areas are critical for recognition memory (Eichenbaum, Yonelinas, & Ranganath, 2007; Squire, Wixted, & Clark, 2007; Murray, Bussey, & Saksida, 2007), the role of the hippocampus in this form of memory has been somewhat more controversial.

In humans, recognition memory is usually assessed by presenting the subject with a list of words for example and, after a short delay, either asking a subject to verbally recall the words in the list as well as sometimes recollecting something associated with the word (recollection), or re-presenting the words one by one and asking the subject to answer whether the word was in the list or not (Yes/No recognition). However, these verbal recognition tasks cannot be used to tax recognition memory in nonhuman species or pre-verbal human infants. To circumvent this problem, researchers have designed three

non-verbal paradigms: the memory span task, the delayed nonmatching-to-sample task (DNMS, or matching-to-sample, DMS) and the visual paired comparison (VPC) task (also known as "preferential looking"), of which the latter two will be the focus of this discussion.

In the DNMS task, the animal initially learns the rule that, when presented with a novel object paired with an object that he has seen recently, the novel object must be displaced in order to obtain a reward, thus involving intentional encoding of the object information (Nemanic, Alvarado, & Bachevlaier, 2004). After attaining a learning criterion, recognition memory is assessed by either increasing the delay between the sample presentation and the choice test, or increasing the number of objects to be remembered. VPC on the other hand takes advantage of an animal's natural preference for looking towards a novel stimulus over a familiar one and does not require the animal to learn any rules, thus involving incidental encoding of the object. This task was originally used by Fagan (1970) to test recognition memory in human infants and has been imported in the animal literature more recently. In VPC, subjects are first familiarized by looking at a sample object and, after varying delays, the sample object is presented together with a new object and subjects' preference for looking towards the novel stimuli is the measure of recognition. These two tasks have been used to investigate the role of the hippocampus and adjacent cortical areas in recognition memory in humans, monkeys and rodents. Superficially, both tasks should require similar cognitive processes for performance, and damage that impairs performance on one of the two tasks should produce a similar impairment on the other. Damage to the PRh affects performance on both tasks, which supports this prediction, however this is not the case

for damage to the hippocampus, which yields no, or moderate, impairment on DNMS but severe recognition loss on VPC (see for review Baxter and Murray, 2001; Bachevalier, Nemanic, & Alvarado, 2002; Nemanic et al., 2004).

Hippocampus

Using DNMS, early studies demonstrated that combined lesions of the amygdala, hippocampus and surrounding cortex severely affected recognition memory. However, damage restricted mostly to the hippocampus resulted in much milder recognition deficits in monkeys (Mishkin, 1978; Bachevalier and Mishkin, 1994; Murray and Mishkin 1983; 1984; 1985; Gaffan, 1974; Zola-Morgan and Squire, 1986; Zola-Morgan, Squire, Amaral, & Suzuki, 1989) and rodents (Mumby, Gaskin, Glenn, Schramek, & Lehmann, 2002; Gaskin, Tremblay, & Mumby, 2003). Later studies demonstrated that selective neurotoxic lesions of both the hippocampus and the amygdala, sparing the cortical areas, resulted in no recognition memory loss at any delay of the DNMS (Murray and Mishkin, 1998). Yet, this lack of impairment is still controversial, given that a recent set of studies in monkeys (Beason-Held, Rosene, Killiany, & Moss, 1999; Zola, Squire, Teng, Stefanacci, Buffalo, & Clark, 2000), rats (Dudchenko, Woods, & Eichenbaum, 2000; Hampson, Simeral, & Deadwyler, 1999) and humans (Reed and Squire, 1997) shows that hippocampal lesions produce recognition memory impairments that are most severe at the longest delays tested or when a distracter is introduced during the delay (Nemanic et al., 2004).

By contrast, all studies using the VPC task in humans and non-human primates to measure recognition memory have found profound loss of novelty preference following selective hippocampal damage. Thus, adult monkeys with ibotenic lesions of the

hippocampus were impaired at delays greater than 30s in VPC (Nemanic et al., 2004) or at delays of 10s and 10min (Zola et al., 2000), even though the same animals showed no, or little recognition deficit in DNMS. Similarly, patient YR, who has circumscribed damage to the hippocampus, performed in the normal range in a Yes/No recognition task (similar to DNMS) but showed no preference for novelty in the VPC task (Holdstock, Mayes, Roberts, Cezayirli, Isaac, O'Reilly, et al., 2002; Pascalis, Hunkin, Holdstock, Isaac, & Mayes, 2004). However, findings from rodents using the novel object preference task (NOP), an analog of VPC, have been less consistent with some studies reporting an impairment similar to that of primates (Clark et al., 2000; Broadbent et al., 2004; Gould et al., 2002) and others reporting no impairment (Forwood et al., 2005; Gaskin et al., 2003; Mumby et al., 2002, 2005; Winters et al., 2004; O'Brien et al., 2006; Hammond et al., 2004). The inconsistent findings between studies is most likely due to variations of the NOP task parameters, including delay, length of time to familiarize to the object and the amount of feature ambiguity between the stimuli (Zeamer and Bachevalier, 2009b).

Given the similarities between the two recognition tasks, the different outcomes of hippocampal lesions suggest that recognition memory ability measured by the VPC and DNMS tasks might differentially engage the hippocampal formation. It has been suggested that, with DNMS, based on the ease of object discriminability, the motivation for a food reward, and the rule guided responses required**,** an animal with hippocampal lesions could use alternative strategies to maintain the memory of an object for short delays, despite its recognition memory deficit. By contrast, with VPC, because of the passive encoding of the image, it may be more ecological for the animal to form a

conjunctive representation of the image and the event in the likelihood that the stimulus could later be relevant (Nemanic et al., 2004). It has also been suggested that VPC may not measure explicit memory at all, but may instead be more implicit, and preference for novelty is seen because of repetition suppression, or a decrease in neuronal firing as the stimulus becomes familiar (Snyder, 2007). However, Manns and colleagues found that the performance of healthy subjects on VPC correlated with subsequent confidence ratings of recognized items 24hrs later, and that a correlation was not found between priming and subsequent recognition (Manns et al., 2000), suggesting that subjects tested on VPC use explicit, and not implicit encoding.

Thus, it is possible that the hippocampus may be preferentially involved in some recognition tasks and not others. Indeed, other studies suggest that the hippocampus mediates at least some processes necessary for recognition memory. First, neuroimaging studies have shown that the hippocampus is activated when human subjects performed recognition memory tasks (Danckert, Gati, Menon, & Kohler, 2007; see for review Cohen, Ryan, Hunt, Romine, Wszalek, & Nash, 1999), and electrophysiology studies in rodents have demonstrated that, after repeated presentations of an object (i.e. as an object becomes familiar), elevated levels of *c-fos* are found in the hippocampus (Zhu, Brown, McCabe, Aggleton, 1995).

To sum, there is still much debate in the literature on the participation of the hippocampus in recognition memory processes (see Squire et al., 2007). By contrast, the contribution of the perirhinal cortex in recognition memory has received mounting support in the last decade (for review see Murray & Richmond, 2001; Eichenbaum et al., 2007).

Perirhinal Cortex

Evidence for a role of the perirhinal cortex in recognition memory came first from lesion studies in monkeys. Thus, monkeys with perirhinal lesions alone (Buffalo, Ramus, Clark, Teng, Squire, & Zola, 1999; Nemanic et al., 2004), or in combination with the entorhinal cortex (Gaffan & Murray, 1992, Meunier, Bachevalier, Mishkin, & Murray, 1993; used DMS: Baxter & Murray, 2001) or the parahippocampal cortex (Zola-Morgan et al., 1989) are severely impaired in DNMS as soon as the delays are extended to a few seconds. Similar results have been found with the VPC task (Nemanic et al., 2004) and are supported by lesion studies in rodents as well (Mumby & Pinel, 1994; Mumby, Piterkin, Lecluse, & Lehmann, 2007). Electrophysiological and gene expression studies also provide evidence for a role of the perirhinal cortex in recognition memory. Thus, perirhinal neurons in primates have been shown to decrease their firing pattern as the stimulus becomes familiar (repeated presentation of a novel stimulus) (Xiang & Brown, 1998; Brown & Xiang, 1998). Similarly, perirhinal neurons in primates also show decreased firing when a familiar stimulus is presented during the test phase of DMS, but firing increased if the object was presented again later as the study image (Hölscher, Rolls, & Xiang, 2002). Also, the cellular expression of Fos protein in the perirhinal cortex increased more in rats that were viewing a series of novel objects than in rats that were viewings familiar objects or no objects at all, (Zhu et al., 1995; Zhu, McCabe, Aggleton, & Brown, 1996; 1997, Wan, Aggleton, & Brown, 1999). Lastly, using 2-deoxyglucose (2-DG), Davachi and Goldman-Rakic (2001) showed increased metabolic rates in the perirhinal cortex of monkeys that were trained on DMS.

Additional evidence for the involvement of the perirhinal cortex in recognition memory comes from a human patient, NB. Due to temporal lobe epilepsy, Patient NB had a resection of part of her temporal lobe that included the perirhinal cortex but spared the hippocampus. She was given several different tasks, including the remember/know paradigm in which a subject must identify whether an image is new or old; and if he/she answers old, the subject must say whether they "remember" seeing that image before, or whether they simply "know" they have seen it, but don't actually remember the event. In this task, a "remember" response is supposed to reflect recollection, which is hippocampal dependent, and a "know" response is supposed to reflect familiarity, which is perirhinal dependent. While her responses showed that she had intact recollection, she was impaired when making a familiarity response (Bowles, Bellgowan, Mirsattari, Pigott, Parrent, Pruessner, et al., 2007), giving further support to the idea that the perirhinal cortex plays a role in recognition memory.

There have also been several imaging studies in humans that suggest that the perirhinal cortex is involved in object recognition memory. Patients with damage to the medial temporal lobe that included the perirhinal cortex showed intact recognition memory on a yes/no recognition task with delays of 0 or 2 s, but were impaired at delays of 6 s and greater. In addition, at delays of 25 s and greater, they performed worse than amnesic patients with intact perirhinal cortices (Buffalo, Reber, & Squire, 1998). Furthermore, increased activation in the perirhinal cortex is seen when normal subjects were shown an array of five objects, and then given a retention test in which one of the objects was replaced by a novel object (Pihlajamaki, Tanila, Kononen, Hanninen,

Hamalainen, Soininen, et al., 2004) or when subjects claimed that the image was new to them as compared to images that were familiar to them (Danckert et al., 2007).

Therefore, based on the behavioral evidence from DNMS and VPC, as well as evidence from human patients, neuroimaging, electrophysiology and gene expression studies, there is a general consensus that the perirhinal cortex is important for object recognition memory.

Current Theories

The evidence presented above suggests that, while the perirhinal cortex and hippocampus are both involved in recognition memory processes, they may each be involved in different aspects of those processes. Thus, there are several theories that have attempted to describe a dissociation of recognition memory processes between these two structures. Brown and Aggleton (2001) originally argued for a distinction between recognition memory processes, suggesting that the perirhinal cortex supports memory judgment based on familiarity and is required when subjects have to judge how recently a stimulus had been presented. By contrast, the hippocampus supports memory judgment based on recollection, not only of the object itself but also of the context (time and space) in which this object has been presented. This idea was expanded in the neural network-based Complementary Learning Systems model of recognition memory developed by O'Reilly and colleagues (Norman and O'Reilly, 2003; O'Reilly and Norman, 2002; O'Reilly and Rudy, 2001). The model posits that the hippocampus is responsible for binding together the sensory features involved during an episode and creating a unitary representation of that episode by the use of relatively non-overlapping, or "pattern

separating" representations. These representations are distinct for each stimulus and allow the hippocampus to learn rapidly without suffering from interference (see also McClelland, McNaughton, & O'Reilly, 1995; O'Reilly and Rudy, 2001). By contrast, the medial temporal cortex, including the perirhinal cortex, integrates information across many experiences to arrive at a general representation of the environment. It learns slowly, and assigns similar neural representations to similar stimuli, allowing it to generalize to novel stimuli based on their similarity to those that were previously encountered. Therefore, recollection may depend more on the hippocampus and familiarity may rely more on the surrounding cortex.

A related model has been proposed by Eichenbaum and colleagues (for review see 2007), who argue that the perirhinal cortex supports familiarity by encoding each specific stimuli separately and then compares a novel stimulus to those previously stored item representations to see if there is a match. The hippocampus, however, supports recollection processes, but not familiarity processes, by associating the stimulus with the context in which it occurred. Finally, Squire and colleagues (for review see 2007) argue that the perirhinal cortex and hippocampus don't actually process familiarity and recollection processes respectively, but can be dissociated according to the strength of the memory trace they support. Thus, while the perirhinal cortex may maintain weak memory traces, the hippocampus may maintain strong memory traces.

To sum, there is still heated debates on the precise processes supported by the perirhinal cortex and the hippocampus in recognition memory. However, we have gained enough knowledge to begin to look at how these different medial temporal lobe structures

develop during ontogeny and when they may be available to support recognition memory processes.

Development of Medial Temporal Lobe Structures

While there are currently very few studies detailing the morphological, neurochemical and functional maturation of the medial temporal lobe structures, the available data indicate that the maturation of the hippocampal formation may lag behind that of the medial temporal cortical areas (see for reviews Alvarado & Bachevalier, 2000; Zeamer et al., 2009a).

In monkeys, neurogenesis of the CA fields as well as the dentate gyrus occurs mostly during prenatal life, but morphological changes (synaptogenesis), fine-tuning of the synaptic connections, myelination as well as neurochemical changes persist for several years after birth in the hippocampus (see for review Alvarado & Bachevalier, 2000; Lavenex, Lavenex, & Amaral, 2007; Seress & Ribak, 1995a; 1995b). This postnatal maturation of the hippocampus is also supported by a recent longitudinal structural neuroimaging study that revealed an increase in overall hippocampal volume as well as changes in the ratio of gray to white matter from birth to 1 year of age in monkeys (Machado, Babin, Jackson, & Bachevalier, 2002). In addition, the volume of the dentate gyrus remains relatively the same between 3 weeks and 3 months of age, but nearly doubles by adulthood (Lavenex et al., 2007). Similarly, neurogenesis in the dentate gyrus is approximately 80% complete at birth, but nearly 20% of neurons are added postnatally (Lavenex et al., 2007). In addition, in the second half of the first postnatal year, CA3 neurons increase in number and in size, and their spines increase in complexity (Seress &

Abraham, 2008). Throughout the first postnatal year, synapses from axons of dentate neurons contacting the dendrites of the CA3 cells (mossy fiber pathway) are formed and there is an increase in the myelination of hippocampal afferent and efferent fibers. However, neurons in the CA1 region show early expression of neurotensin (NT) and may have established connections with ERh by midgestation in monkeys, as suggested by the presence of NT reactive terminals in that region at that stage (Berger, Alvarez, & Goldman-Rakic, 1993; Berger & Alvarez, 1994). A similar protracted postnatal development of the hippocampus has been described in humans (Seress & Abraham, 2008) in which reciprocal connections between CA1 and ERh can be found by 19 weeks gestation, that is at a time when the perforant path projections (ERh to dentate gyrus) are sparse (Hevner & Kinney, 1996).

For both ERh and PRh, most of the neurogenesis occurs prenatally, yet several morphological and neurochemical changes occur in the first few months postnatally. The rhinal sulcus, which divides ERh from PRh, is still only a small indent on the cortical surface by the last quarter of gestation (Berger & Alvarez, 1994), but by birth, the cytoarchitectonic and chemoanatomical characteristics of PRh and ERh in the primate can be clearly identified and appear adult-like (Berger & Alvarez, 1994). Although afferent projections from the entorhinal cortex to the dentate gyrus are present by 3 weeks of age (Lavenex et al., 2007), the dentate gyrus, as described above, continues to mature during the first postnatal year.

Thus, while the direct connection between the ERh and the CA1 field (by-passing the dentate gyrus) seems to be present in the first few months of life, the perforant path conveying inputs from the ERh to the dentate gyrus, and consequently the trisynaptic

circuit of the hippocampus, may mature progressively during the first year of life. Given this developmental time course, Alvarado and Bachevalier (2000) have proposed that some form of memory processes supported by the ERh-CA1 pathway may emerge earlier in development than memory processes supported by the trisynaptic pathway.

Development of Recognition Memory Processes

In contrast to the adult literature reviewed above, in which the understanding of the biological foundations of memory has been substantial over the last 2 decades, in the developmental literature the search has just started. This lack of attention to the study of the neural bases of memory in development is not due to a lack of interest in developmental changes in memory per se. Indeed a tremendous amount of research mainly in the developing human has provided evidence of developmental patterns in memory functions.

One of the first attempts to correlate brain maturation and memory development emerged from a distinction between the development of procedural versus declarative memory processes in monkeys. Using memory tasks that have been shown to be mediated by different neural circuits in the adult monkeys (e.g. DNMS mediated by the medial temporal lobe structures and concurrent discrimination mediated by a cortical-striatum circuit), Bachevalier and Mishkin (1984) showed that, although 3-month-old monkeys could solve the concurrent discrimination task as efficiently as adults, it is not before 4 months of age that they can begin to solve the DNMS rule and not before 2 years that they can master the DNMS rule as efficiently as adult monkeys (Málková, Bachevalier, Webster, & Mishkin, 2000). In addition, all infant monkeys (3-, 6- and 12-

month-old) showed poorer recognition memory with increasing delays and lists of objects to remember. Similarly, human children do not perform at adult-like levels on the DNMS task until 4-5 years of age, although they can master a concurrent discrimination task at a much earlier age (Overman, Bachevalier, Miller, & Moore, 1996). These findings suggested that recognition memory processes mediated by structures within the medial temporal lobe have a protracted postnatal development in primates. Nonetheless, subsequent studies using the VPC task suggested otherwise.

The development of recognition memory processes as assessed with VPC is known to emerge early in infancy in both humans and non-human primates (Pascalis & de Schonen, 1994; Gunderson & Sackett, 1984; Bachevalier, Brickson, & Hagger, 1993, for review see Rose, Feldman, & Jankowski, 2007). Human infants can show novelty preference as early as 3-4 days of age with either no delay or a delay of 2 minutes (Pascalis & de Schonen, 1994). Similarly, infant pigtail macaques do not show a preference for novelty at one week of age, but can show novelty preference as early as 4 weeks of age, and this preference becomes stronger by thirteen weeks of age (Gunderson & Sackett, 1984). In addition, infant pigtail macaques averaging 86 days old show novelty preference even after a delay of 24 hrs (Gunderson and Swartz; 1985). Thus, with VPC, the data suggest that recognition memory processes emerge early in infancy for both human and non-human primates. This set of data led researchers to conclude that because of their early emergence in ontogeny, recognition memory processes mediated by the hippocampus must be maturing very early in life.

However, until now, few studies have actually attempted to demonstrate whether the hippocampus is truly supporting recognition memory abilities in early infancy. One

of the first studies examined the effects of neonatal lesions of the medial temporal lobe (including not only the hippocampus but also the amygdala and the adjacent cortical areas) on novelty preference in infant monkeys at 5, 15, and 30 days of age using the VPC task and a short delay of 10 sec (Bachevalier et al., 1993). Infants with the neonatal medial temporal lobe lesions did not show novelty preference as compared to controls, suggesting that recognition memory processes associated with the VPC task may indeed emerge early in life. In a second study, adult monkeys that received neonatal lesions of the hippocampal formation, which included the hippocampus and most of the parahippocampal cortex, also showed a lack of novelty preference at delays of 30 sec and longer on the VPC task (Pascalis & Bachevalier, 1999). Nevertheless, because the neonatal lesions in these two studies were not limited to the hippocampus, it is difficult to conclude whether the hippocampus and/or the entorhinal and perirhinal cortex are the neural substrates mediating the early developing recognition processes measured by VPC.

To more precisely investigate whether the hippocampus and/or the perirhinal cortex mediate recognition memory processes measured by VPC in early infancy, we ran a series of developmental studies that form the topic of the present project.

Specific Hypotheses

Given recent evidence suggesting that object recognition memory as measured by the VPC task in adult monkeys is dependent on the integrity of the perirhinal cortex at short delays and the hippocampus at longer delays (Nemanic et al., 2004), and our current knowledge on the anatomical development of these structures in primates, we

hypothesize that the perirhinal cortex, which seems to be mature shortly after birth, could be sufficient to support early developing recognition processes, but that the hippocampus, which has a more protracted development, could become engaged in such processes at a later age. To test this proposal, we designed two longitudinal studies in monkeys using the visual paired comparison task as a measure of incidental recognition memory.

Experiment 1

The goal of this first experiment was twofold. First, to investigate the normal development of recognition memory in non-human primates, as well as the contribution of the hippocampus in the development of these memory processes, infant monkeys were prepared with either sham surgeries (Neo-C) to serve as controls, or with neonatal neurotoxic lesions of the hippocampus (Neo-Hibo). Given what is known about the maturation of the hippocampal formation, monkeys were tested at 1.5, 6, 18 and 48 months of age on the VPC task using color pictures of objects. In addition, to allow comparisons with scores previously reported in animals that had received the same lesions in adulthood, at each age delays varied from 10, 30, 60 and 120s. Second, given that monkeys with adult lesions of the hippocampus are impaired on VPC only when stimuli share several overlapping features (Zeamer and Bachevalier, 2009b), animals in Groups Neo-C and Neo-Hibo were also tested at 48 months using the same sets of stimuli as in the adult lesion study. These sets of stimuli consisted of either black and white stimuli that varied in shape, texture and gradients of black and white (BW), or black and white stimuli that were as similar as possible, taken from the same category (bugs, tables, phones etc.) and had many overlapping features (BW-OF).

Experiment 2

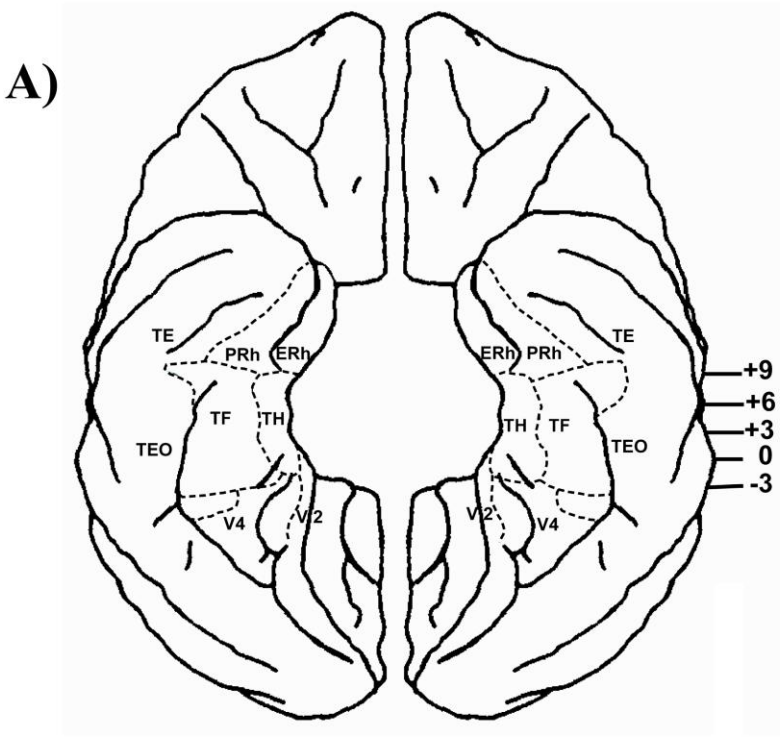
The goal of this experiment was to investigate whether the perirhinal cortex supports early developing recognition memory processes. Infant monkeys were prepared with neonatal neurotoxic lesions of the perirhinal cortex (Neo-PRh). They were tested at 1.5, 6 and 18 months of age on VPC with delays of 10, 30, 60 and 120s using color pictures in order to make comparisons with sham-operated controls and animals with neonatal lesions of the hippocampus from Experiment 1. The monkeys in Group Neo-PRh have not reached adulthood, so have not yet been tested at 48 months.

Figure Legend

Figure 1: Anatomical organization of the medial temporal lobe.

Drawing of the ventral view of a macaque brain with cytoarchitectonic cortical areas delineated by dashed lines (Zeamer et al., 2009a). B) Schematic diagram of medial temporal lobe inputs and outputs from the hippocampus. The arrow width relates to the strength of the connections (Suzuki and Amaral, 1994a). Abbreviations: CA1 and CA3 – Ammonic subfields of the hippocampus; DG – dentate gyrus; ERh – entorhinal cortex; PRh – perirhinal cortex; Sub – subiculum; TE, TEO, TF and TH – cytoarchitectonic fields as defined by von Bonin and Bailey, 1947; V2 and V4 – secondary (extrastriate) visual cortex.

Figure 1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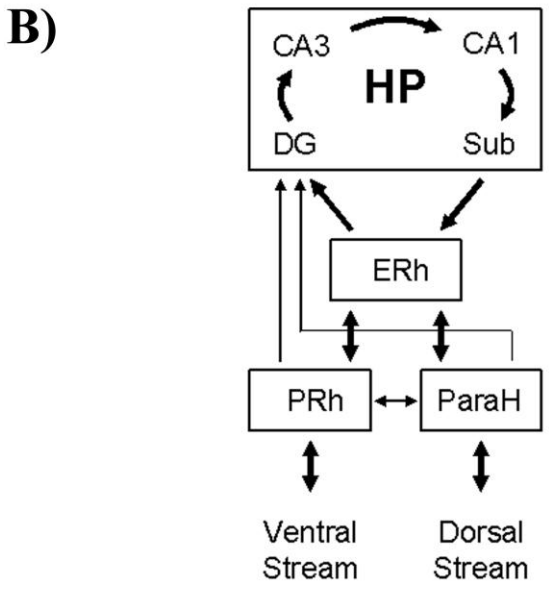
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The development of object recognition memory in rhesus macaques with neonatal lesions of the hippocampal formation

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Abstract

To systematically investigate the development of recognition memory as assessed by the visual-paired comparison task (VPC), this study longitudinally examined object recognition memory in monkeys (*Macaca mulatta*) with neonatal sham operations and animals with neonatal lesions of the hippocampus. All subjects were tested on VPC with delays from 10 s to 120 s at 1.5, 6, 18 and 48 months of age. In addition, because findings from adults tested on VPC show that the magnitude of recognition memory impairment varied with the types of stimuli used (e.g. better performance with color pictures than with pictures of black/white objects with overlapping features, BW-OF), the subjects were also tested on VPC with more difficult black and white stimuli at 48 months. Additionally, performance scores at 48 months with both color and BW-OF pictures were compared to those obtained with animals that had received lesions of the hippocampus in adulthood and tested in the same way. The findings reveal that incidental recognition memory is present early in normal controls, but it becomes delay-dependent by 18 months of age. Animals with neonatal lesions of the hippocampus performed weaker than controls only at the longest delay of 120 s at 18 months, but their performance was abolished with the same delay and stimuli with many overlapping features. Finally, while animals with neonatal lesions performed similarly to those with adult lesions with color stimuli, they showed stronger novelty preference with the shorter delays for stimuli with overlapping features, suggesting some sparing of function. These findings also suggest that the hippocampus may not be involved in incidental recognition memory until around 18 months of age, at which point it begins to interact with the cortical areas to support

this function. Thus, in the absence of a functional hippocampal formation, the medial temporal cortical areas can maintain normal recognition memory performance.

Introduction

The contribution of the hippocampus to recognition memory processes remains under intensive debate in the literature (see for review Squire et al., 2007; Eichenbaum et al., 2007; Murray et al., 2007). Nevertheless, selective hippocampal damage in primates has been shown to severely impact recognition memory processes when assessed with the visual paired comparison task (VPC), a task which measures incidental recognition memory processes. Thus, adult monkeys with selective hippocampal lesions showed a profound loss of novelty preference at delays greater than 10s (Zola et al., 2000) or 30s (Nemanic et al., 2004) with black and white stimuli, or profound impairment when using stimuli with overlapping features (Zeamer and Bachevalier, 2009b). Similarly, patient YR who has circumscribed damage to the hippocampus, showed novelty preference at delays of 0 s but not at longer delays of 5 or 10 s when tested in the VPC task (Pascalis et al., 2004). Novelty preference is also altered by damage to the medial cortical areas, although this impairment emerged at shorter delays, i.e. 10 s for damage to the perirhinal cortex and 30 s for damage to parahippocampal areas TH/TF (Nemanic et al., 2004). These findings suggest that both the hippocampus and the medial temporal cortical areas contribute differently to recognition memory processes. Nevertheless, the contribution of these medial temporal structures to the emergence of recognition memory processes during development has never been systematically studied.

Due to its incidental nature, the VPC task has been a task of choice to study recognition memory development in primates, including humans (Fagan, 1970). In humans, recognition memory measured by VPC emerges as early as 3-4 days of age with either no delays or delays of 2 minutes (Pascalis & de Schonen, 1994). Similarly, infant

pigtail macaques do not show a preference for novelty at one week of age, but can show novelty preference as early as 4 weeks of age, and this preference becomes stronger by thirteen weeks of age (Gunderson & Sackett, 1984, Bachevalier et al., 1993). Because adults with hippocampal damage are impaired on this task, it has been argued that the presence of hippocampal-dependent recognition memory processes in early infancy indicates that the hippocampus is functional very early in life (Bachevalier, 1990).

However, there are some serious problems with this assertion. First, because recognition memory processes as measured by VPC are not dependent only on the integrity of the hippocampal formation but also on that of the medial temporal cortical areas, it is possible that the presence of novelty preference at a young age could be supported by the cortical areas in the absence of a functional hippocampus. Second, because the effects of selective damage to the hippocampus and the cortical areas have been shown to alter recognition memory at different delays, it will be critical to know how novelty recognition at different delays varies across development. There have been no studies that have systematically followed the development of recognition memory from infancy through adulthood. Furthermore, there are only two studies in monkeys that have actually attempted to demonstrate whether the hippocampus is truly supporting recognition memory abilities in early infancy. One study (Bachevalier et al., 1993) reported a lack of novelty preference at 5, 15, and 30 days of age using the VPC task and a short delay of 10 sec in infant monkeys that had received extensive neonatal damage to the medial temporal lobe. In a second study, adult monkeys that received neonatal lesions of the hippocampal formation also showed a lack of novelty preference at delays of 30 sec and longer on the VPC task (Pascalis & Bachevalier, 1999). However, in both

of these studies the neonatal lesions were not restricted to the hippocampus, but also involved the adjacent cortical areas for the former and parahippocampal cortical areas for the later. Thus, although the authors indicated that the medial temporal lobe structures appeared to support recognition memory early in infancy, it is still premature to conclude that the hippocampus is the critical structure mediating the early developing recognition processes measured by VPC.

To more precisely examine the maturation of recognition memory processes in normal infant monkeys across development and the contribution of the hippocampus to the development of recognition memory, the present study had four aims. The first was to systematically follow performance on the VPC task from infancy through adulthood, using color stimuli and varying delays. The second was to assess the effects of neonatal hippocampal lesions on recognition memory processes through development. The third was to compare the effects of neonatal hippocampal lesions to those following damage to the hippocampus in adulthood (Zeamer and Bachevalier, 2009b) and to assess whether any sparing of function may have occurred as a result of the early hippocampal damage. Finally, the fourth aim was based on the recent demonstration in adult monkeys that the magnitude of recognition impairment following hippocampal damage varied depending on the type of stimuli used, i.e. no impairment with color stimuli but weaker novelty preference with black and white stimuli with overlapping features (Zeamer and Bachevalier, 2009b). Thus, this aim compared the effects of stimulus types on the magnitude of recognition deficits found after neonatal hippocampal lesions when the animals reached adulthood. Preliminary reports of the findings were published in

abstract form (Resende, Chlan-Fourney, & Bachevalier, 2002; Zeamer & Bachevalier, 2006; Zeamer, Resende, Heuer, & Bachevalier, 2005).

Methods

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Texas at Houston where this study began and of Emory University where it was completed. All procedures were kept constant between the two institutions.

Subjects

The subjects were eleven full-term infant rhesus monkeys (*Macaca mulatta*), 6 males and 5 females, acquired from the MD Anderson Cancer Center Science Park breeding facility. They arrived in 3 cohorts of 4 animals, approximately a year apart. Between 1 and 4 days after birth, they were brought from MD Anderson Cancer Center Science Park (Bastrop, TX) to the primate nursery at MD Anderson Cancer Center (MDA, Houston, TX) where they were hand fed a diet of infant Similac formula. They were nursery reared according to procedures developed by Sackett and colleagues (2002) that allow normal survival and growth as well as species-specific social skills and pregnancy outcomes in monkeys. These procedures include daily social interactions with peers, intensive human care, and cognitive testing (for additional rearing details, see Goursaud & Bachevalier, 2007). At 10-12 days, three males and 3 females received a sham operation (Group Neo-C), and 3 males and 2 females received MRI-guided neurotoxic lesions of the hippocampus (Group Neo-Hibo), bilaterally. Starting around 8

months of age, all animals were fed jumbo primate chow (Lab Diet #5037, PMI Nutrition International Inc., Brentwood, MO) and fresh fruit daily. When the three cohorts were 3, 2 and 1.5 years old, respectively, they were moved to Yerkes National Primate Research Center (Atlanta, GA).

Surgical Procedures

Pre- and postsurgical Magnetic Resonance Imaging (MRI) scans

Two neuroimaging sessions were used, one immediately prior to surgery and the other 5-8 days post-surgery (Málková, Lex, Mishkin, & Saunders, 2001; Nemanic, Alvarado, Price, Jackson, & Bachevalier, 2002). The pre-surgical MR images were used to determine the injection site and the post-surgical MR images were used to estimate lesion extent. All animals received the pre-surgical scans, but only those in Group Neo-Hibo were scanned post-surgery. For each MR scan, the subjects were placed in an induction box saturated with isoflurane gas, intubated and maintained under isoflurane gas (1.0-3.0%, v/v, to effect) throughout the procedure, and placed in the stereotaxic apparatus. Images were acquired with a 5-cm surface coil using a GE Signa 1.5 Tesla Echo Speed scanner (GE Medical Systems, Milwaukee, WI at UT-Houston). After a short sagittal scout (T1-weighted spin-echo sequence, echo time (TE) = 11 ms, repetition time (TR) = 450 ms, contiguous 4 mm sections, 12 cm field of view (FOV), 256 x 256 matrix) to align two different MR sequences in the coronal plane. The first was a 3D T1-weighted fast spoiled gradient (FSPGR)-echo sequence (TE = 2.6 ms, TR = 10.2 ms, 25° flip angle, contiguous 1 mm sections, 12 cm FOV, 256 x 256 matrix) that was to calculate the coordinates of each injection site along the length of the hippocampal

formation. The second set was a Fluid Attenuated Inversion Recovery (FLAIR) sequence (TE = 140 ms, TR = 10000 ms, inversion time (TI) = 2200ms, contiguous 3 mm sections, 12 cm FOV, 256 x 256 matrix) that was repeated three times, offset by 1mm posteriorly and was used post-surgery to locate the region of hypersignals around the injection sites indicative of edema caused by neurotoxin-induced cell death. These hypersignals were used to estimate the extent of lesions, which was subsequently confirmed by an additional MRI session approximately one year after surgery in animals of Group Neo-Hibo, using T1 high resolution MRI images. These images were used to estimate the percent reduction of the hippocampal formation bilaterally (Nemanic et al., 2002).

Surgery

All surgical procedures were performed under deep anesthesia using aseptic techniques. Throughout surgery the animal was maintained on Isofluorane gas (1.0 – 2.0%, v/v, to effect), an IV drip containing dextrose and 0.45% sodium chloride was used to maintain normal hydration, a heating pad was placed under the animal to prevent hypothermia, and vital signs were monitored until the monkey fully recovered from anesthesia.

The scalp was shaved, the skin was disinfected with Nolvasan solution, and the subject was injected with a long lasting local anesthetic (Marcaine 25%, 1.5 ml) along the incision line. A midline longitudinal incision was made and the skin and connective tissue (galea) were gently retracted. Craniotomies were then made bilaterally above the injection sites and Bone wax (Ethicon, Inc., Somerville, NJ; 2.5g size) was applied to prevent excessive bleeding. The dura was opened and 2 Hamilton syringes filled with ibotenic acid (Biosearch Technologies, Novato, CA, 10 mg/ml in PBS, pH 7.4) and held

by Kopf electrode manipulators (David Kopf Instruments, Tujunga, CA) were lowered simultaneously at each injection site in the hippocampus of each hemisphere. At the level of the uncus two injection sites were placed 4mm from each other in the medio-lateral plane and 5 injection sites were placed along the body of the hippocampus separated by 2mm. A total of 5.0µl ibotenic acid was injected bilaterally, at a rate of 0.4 µl per minute, followed by a three-minute delay to permit diffusion of the neurotoxin and minimize its spread up the needle track during retraction. Once all injections were completed, the wound was then closed in anatomical layers and the subject was removed from isoflurane and recovered in the surgical suite until it could breathe on its own. The subject was then moved back to the nursery and placed in an incubator ventilated with oxygen until the next day. Beginning 12 hours before surgery and lasting 7 days after surgery, dexamethazone sodium phosphate (0.4 mg/kg, i.m.) and Cephazolin (25 mg/kg, i.m.) were given to reduce edema and prevent infection, respectively. In addition, acetaminophen (10 mg/kg, p.o.) was given four times a day for 3 days after surgery for relief of pain.

The sham lesions followed the same procedures with the exception that after the dura opening, there were no needle penetrations and the tissue were immediately closed in anatomical layers.

MRI-based lesion evaluation

Because all subjects are still participating in other cognitive experiments, no histological evaluations are available. Therefore, estimation of lesion extent is provided using the FLAIR images obtained one week post-surgery and the T1 high-resolution images obtained one year post-surgery (for details see Málková et al., 2001; Nemanic et

al., 2002). Briefly, the hypersignals on the FLAIR images were visually identified and plotted onto the corresponding coronal section of a normal monkey brain at 1-mm intervals, and were then imported into ImageJ® to measure the surface area (in pixels^2) of hypersignals within the left and right hippocampal formation (including all CA fields, dentate gyrus, and subicular complex) as well as adjacent neural structures (i.e. perirhinal cortex, entorhinal cortex, areas TH and TF on the parahippocampal gyrus and amygdala), if any. Estimated percent hippocampal volume damaged was then calculated by dividing the total volume of hypersignals for the hippocampus on the right and left hemispheres by the normal hippocampal volume. In addition, to estimate the percent reduction of hippocampal volume one year after surgery, each T1 image at 1-mm intervals throughout the entire hippocampus was imported into ImageJ and the surface area of the hippocampus was measured (in pixels^2). Percent reduction was then calculated using the following formula: [100-total H volume remaining/average H volume in normal subject]*100).

Behavioral Apparatus and Stimuli:

Testing was conducted in a sound-attenuated room equipped with a white noise generator to reduce external noise. A TV monitor was positioned on a table at eye level of the animal and a video camera (Sony Digital8 TRV-140) was mounted above the screen and 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. Stimuli were color pictures of variegated objects (trees, animals, tools, etc…) selected from a pool of 800,000 clipart

images (Nova Art Explosion 800,000 Clip Art) and varied in color, shape and texture. For the comparison with monkeys with adult lesions of the hippocampus, we created two more pools of images, one pool that included pairs of black and white (BW) pictures that differed in shape, texture and in gradients of black, gray and white, and a second pool that included pairs of black and white pictures of the same category (tables, chairs, dogs, etc), such that the two stimuli in the pairs shared many overlapping features (BW-OF). All images were delivered to the monitor via a computer controlled by the experimenter.

Pre-Training:

At 1.5 and 6 months of age, the infant was held by an experimenter approximately 40 cm in front of a 19" display screen. At older ages, the monkey was seated in a custom made plexiglass primate chair (Crist Instruments) that fit the animal's size. For 3 days prior to the start of testing, the 1.5-month-old infants were brought into the testing room where they watched a Disney cartoon for an increasing period of time each day (15, 20 and 25 minutes) and at 6 months of age, they received one day of pre-training for 20 minutes. At 18 months of age, the animals were first trained over a period of a few days to enter and acclimate to the primate chair, and were then brought into the testing apparatus to view a Disney cartoon for one day. At 48 months, the subjects were also given one day of acclimation to the testing apparatus prior to the beginning of testing. The subjects were neither food deprived nor water restricted and remained in the testing apparatus for no more than 45 minutes a day.

VPC task:

All monkeys began testing on the visual paired comparison task (VPC) at 1.5 months (range: 1.5-1.7), 6 months (range: 6-6.5), 18 months (range: 18-18.5), and 48 months (range: 44-54 months). Each VPC trial was divided into a familiarization phase and a retention phase (Figure 1). In the familiarization phase, a stimulus appeared and remained in the center of the screen until the monkey spent a cumulative 30 seconds looking at the sample image. After a delay, which varied from 10, 30, 60 and 120 seconds, two 5-s Retention Tests separated by a 5-s delay were given. During these two retention tests, the sample image and a novel image were displayed side-by-side on the screen for 5 seconds after the animal initially looked at one of the two images. The inter-trial interval (ITI) was 30s. The left/right position of the two images was reversed during the Retention Tests, and on the first Retention Test the left/right position of the novel image varied pseudo-randomly. The screen remained black during all delay periods. At each age, ten trials were given at each delay. The animals were given 4 – 10 trials per day and did not receive food treats during the testing period at 1.5 and 6 months of age, but were given mini-M&M's during random ITIs at 18 and 48 months to encourage them to remain focused on the screen. At 48 months only, subjects were shown 10 sets of BW and BW-OF trials with delays between the familiarization length and retention test of 10, 60 and 120 seconds. Color trials were presented first, followed by BW trials, then BW-OF trials.

Data Analysis

For each trial, the videorecordings of the two retention tests were analyzed frame-by-frame to calculate the amount of time the animal spent looking towards the familiar

and the novel stimuli (Pascalis and Bachevalier, 1999). Two observers, each blind to the position of the novel image and to the lesion group of the subject, scored the videotapes independently (inter-observer reliability: pearson r = 0.925). The length of time it took the animal to familiarize to the novel object (Total Familiarization Phase) and total time looking at both stimuli during the retention tests (Total Retention Time) were recorded and analyzed. The percent time looking to the novel stimulus across the two retention tests was also calculated [(time looking at novel / total time looking) X 100)]. All trials in which the total looking time during the two retention tests was less than one second were rejected.

Statistical Analysis

All statistical analyses were carried out using the SPSS 12.0 software. A General Linear Model ANOVA was conducted on the three task parameters (Total Familiarization Phase, Total Retention Time, and Percent Novelty) with a between subject comparison for the Group effect (Group Neo-Hibo and Group Neo-C) and within subject comparisons using repeated measures for the Delay and Age effects. To compare novelty preference scores between monkeys with neonatal lesions and moneys with adult lesions of the hippocampus, A General Linnear Model ANOVA was conducted on Percent Novelty with between subject comparisons for Group effect and within subject comparisons for Stimulus type and Delay. When sphericity was not assumed, a Huynh-Feldt correction was used. Post-Hoc Tukey tests were conducted when group differences reached significance. Additionally, planned comparisons were performed between the control group and the experimental group, using a one-sided Planned Comparison

(Pedhazur, 1982). One-sample t-tests were used to evaluate group differences from chance.

Results

Evaluation of Hippocampal lesion:

Estimates of lesion extent for all five subjects (Table 1) ranged from 33.2 to 87.4% (average: 56.9%) and varied across hemispheres, hippocampal fields and along the anterior-posterior axis. In addition, the percent volume reduction of the hippocampal formation, based on measurements taken from the one-year post T1 images, ranged from 19.1 to 67.0% (average: 48.3% reduction; Table 2). The extent of hypersignals seen on the post-surgical FLAIR images for each case are plotted on matched coronal sections through a normal infant brain and shown on Figure 2. A representative case (Case-Hibo-2) depicting the hippocampal volume reduction seen on the one-year post-surgical T1 scan is illustrated in Figure 3.

Two cases (cases Neo-Hibo-2 and 3; Figure 2) received extensive bilateral lesions, with more extensive damage on the right than on the left (Table 1), resulting in more than 50% overall volume reduction of the hippocampus (Table 2). For both cases, sparing mostly occurred in the left uncus and in the medial part of the caudal most portion of the left hippocampus (see Fig. 2, levels 0 to -9). Extent of hippocampal lesions in the three remaining cases (cases Neo-Hibo-1, 4 and 5) were more unilateral, with extensive damage on one side (> 60%) but moderate to mild damage on the other (< 21%). Case Neo-Hibo-1 had sparing along almost the entire length of the right hippocampus, since the neurotoxic injections reached the most lateral part of the hippocampus (see Fig. 2, level -9). For case Neo-Hibo-4, damage to the left hippocampal formation was restricted

to the lateral part of the hippocampus (see Fig. 2, levels 0 and -6). For case Neo-Hibo-5, damage on the left was located in the uncus and lateral portions of the hippocampus (see Fig. 2, levels 0 and -6). It is important to note that, although the volume of damage to the left hippocampus was mild in both cases Neo-Hibo-4 and Neo-Hibo-5, the damage included the CA2 and CA1 fields of the hippocampus. This mild damage in fact disrupted the normal functioning of the trisynaptic hippocampal circuit as well as the direct entorhinal-CA1 pathway. Overall reduction in volumetric hippocampal measures taken on post-surgical T1 images at 1 year of age ranged from 19.1% to 56.6 % in these three cases.

For all cases, unintended damage to adjacent structures was minimal and restricted to 6.5% damage to areas TH/TF and 2.5% damage to the amygdala (see Table 1).

Normal development of object recognition memory

Data for the sham-operated controls were first analyzed separately to assess the development of object recognition memory. There were no effects of age or delay on Total Familiarization Time or Total Retention Time for Group Neo-C (all ps > .05). However, these factors had a differential effect on the magnitude of novelty preference in Group Neo-C (Table 3). Overall, novelty preference was above chance level at all ages and delays. As shown in Figure 4, novelty preference became stronger from 1.5 to 6 months of age, and remained stable through 48 months (Age effect: $F(3,15) = 5.23$, $p < .05$). In addition, there was a marginal Age by Delay interaction ($F(9,45) = 1.79$, $p = .09$), indicating that a delay-dependent effect emerged at some ages and not others. Separate ANOVAs at each age showed that, at the tow youngest ages, novelty preference was

stable across all delays, whereas at 18 and 48 months, novelty preference became significantly weaker with increasing delays (18 months: $F(3,15) = 3.79$, $p < .05$; 48 months: $F(3,15) = 3.377$, $p < .05$). While the main effect was not significant at 18 months, planned comparisons revealed that novelty preference scores were greater at 10-s delay than at 120-s delay ($p < .05$). In addition, at 48 months novelty preference scores were greater at 10-s, 30-s and 60-s delays than at 120-s delay (ps = .016, .058, .068, respectively).

Effects of neonatal hippocampal lesion

Comparisons between Group Neo-Hibo and Group Neo-C revealed no Group, Age or Delay effects on Total Familiarization Time (all ps > .05). By contrast, for Total Retention Time, the Age effect was significant ($F(3,27) = 3.30$, $p < .05$), indicating that the two groups looked longer at the two stimuli at 1.5 and 6 months of age (Mean ± SEM: 7.06 ± .10 and 6.62 ± .22, respectively) than at 18 and 48 months (Mean ± SEM: 5.86 ± .20 and 5.66 ± .30, respectively; all ps < .05, except 6 vs. 18 months, $p = .07$). However, the two groups did not differ ($p > .05$).

As with Group Neo-C, manipulation of both Age and Delay had a differential effect on the magnitude of novelty preference in Group Neo-Hibo, as revealed by no significant effect of Group ($F(1,9) = 2.16$, $p > .1$) but significant effects of Age and Delay ($F(3,27) = 12.78$, $p < 0.001$ and $F(3,27) = 6.91$, $p < .001$, respectively). As shown in Figure 5, novelty preference in Group Neo-Hibo remained above chance levels across all ages and delays, became stronger from 1.5 to 6 months and showed a delay-dependent effect at 18 months (Age X Delay: $F(9,81) = 2.94$, $p < .01$), but not at 48 months.

Further, to analyze the apparent group differences shown in Figure 5 at 18 months, a separate analysis at this age revealed a Group by Delay effect ($F(3,27) = 3.68$, $p < .05$), indicating that, although novelty preference in the two groups became weaker with increasing delays, this effect was greater in Group Neo-Hibo than in Group Neo-C. The two groups differed at delays of 10 s and 120 s ($ps < .05$). The magnitude of the impairment did not correlate with the extent of hippocampal lesions.

Manipulating Feature Ambiguity

Comparisons between Group Neo-Hibo and Group Neo-C revealed that both groups took longer to familiarize to the Color stimuli than to the BW and BW-OF stimuli (Stimulus effect: $F_{HUYNH-FELDT}(1.69,15.22) = 5.14$, $p < .05$). However, for the Total Retention Time, the two groups did not differ across any stimulus type (Stimulus effect: $F_{HUYNH-FELDT}$ $(1.79,16.15) = .04$, $p > .05$).

Manipulation of both delay and stimulus types had a differential effect on the magnitude of novelty preference in both groups. The Delay effect was significant ($F(2,18) = 18.07$, $p < .001$). Further analysis indicated that for all stimulus types and both groups, novelty preference scores were greater at the shorter delays of 10 and 60 s than at 120 s ($ps < .01$). In addition, as shown in Figure 6A, the two groups showed greater novelty preference for both Color and BW stimuli than with BW-OF stimuli (Stimulus effect: $F(2,18) = 13.70$, $p < .01$; Color > BW-OF, $p < .001$, BW > BW-OF, $p < .05$). The analysis also revealed a significant Group by Stimulus type by Delay interaction [$F(4,36) = 3.07$; $p < .05$]. To determine the significance of this interaction, Group by Delay ANOVAs were run at each of the stimulus types. These analyses indicated that there was

no reliable group differences and no Group by Delay interaction for the color and BW stimuli, whereas for the BW-OF stimuli the Group by Delay interaction reached significance [$F(2,18) = 4.13$, $p < .05$]. As shown in Figure 6b, further analysis revealed that for the BW-OF stimuli, Group Neo-Hibo showed weaker novelty preference than Group Neo-C at the longest delay of 120s ($t(9) = 3.53$, $p < .01$). The magnitude of the impairment at the longest delay did not correlate with hippocampal lesion extent.

Effect of neonatal vs adult hippocampal lesion

Data for Group Neo-C was first compared to data from animals that had received their sham operations as adults (Group C; Zeamer & Bachevalier, 2009b). Because animals in Group C not only received their sham-operation as adults but also were mother-reared in a more naturalistic social environment, comparisons between the two groups could help determine the effects of rearing conditions on novelty preference. The two groups did not differ (Group effect: $F(1,7) = 1.16$, $p > .05$), nor were there interactions between Group and Stimulus type or Delay [$F(2,14) = 0.09$, $p > .05$, $F(2,14) = 3.60$, $p > .05$ respectively]. However, as shown in Figure 7A, there was a main effect of Stimulus type [$F(2,14) = 9.16$, $p < .01$], indicating that both groups showed stronger novelty preference with Color than BW and BW-OF stimuli ($ps < .02$), and with BW than BW-OF stimuli ($p = 0.06$). In addition, there was an effect of Delay [$F(2,14) = 4.45$, $p < .05$], indicating that both groups showed stronger novelty preference for 10s and 60s delays than the 120-s delay ($p = .05$ and $p = .07$ respectively).

Comparisons were then made with Group Neo-Hibo and Group H and their sham-operated controls (Groups Neo-C and C, respectively). The four groups did not differ

($F(3,13) = 1.12$, $p > .05$), but there was a Group by Delay interaction ($F(6,26) = 3.40$, $p < .05$) and a marginal Group by Stimulus type interaction [$F(6,26) = 2.21$, $p = .07$]. Although the Group by Stimulus type by Delay interaction did not reach significance ($F(12,52) = 1.36$, $p > .05$), separate Group by Delay ANOVAs for each stimulus type indicated neither Group nor Group X Delay interactions for the Color and BW stimuli, whereas for the BW-OF stimuli, the Group by Delay interaction failed just short of significance ($F(6,26) = 2.37$, $p = .06$). As seen in Figure 7B, although the two control groups performed above chance level at all delays, animals in Group Neo-H showed no novelty preference at the longest delay and differed significantly from both Groups Neo-C and C ($p < .01$ and $p = .06$, respectively). By contrast, Group H showed no novelty preference at both the 60-s and 120-s delays and differed from all groups at the 60-s delay ($ps < .05$) but not at the 120-s delay. Overall, the data indicate that Group Neo-H had stronger novelty preference than Group H at the shortest delays ($p < 0.05$) but not at the longest delay of 120 s.

Discussion

The main goal of this study was to more precisely examine the maturation of recognition memory processes in normal infant monkeys across development, to assess whether the hippocampus was crucial to support these early recognition memory processes, and to compare the effects of neonatal versus adult hippocampal lesions on incidental recognition memory. This study revealed important new findings. First, incidental object recognition memory is present early in life but major delay-dependent changes begin to occur after the first year of life. Second, neonatal hippocampal lesions

transiently weaken incidental object recognition memory for color pictures at long delays, and abolish recognition memory for stimuli with overlapping features at the same long delays. Finally, although the effects of neonatal hippocampal lesions on incidental recognition memory were, for the most part, similar to those of adult hippocampal lesions, the magnitude of the impairment for stimuli with overlapping features was more profound with the adult lesions than with the neonatal lesions, suggesting some potential saving of functions. These findings will be discussed in turn.

Normal development of recognition memory

Infant monkeys with an intact hippocampus showed robust novelty preference as early as 1.5 months of age even at the long delays tested, and this preference for novelty increased by 6 months of age, remaining strong through adulthood. These findings demonstrate that recognition memory is present in early infancy and are consistent with earlier studies in monkeys (Gunderson and Sackett, 1984; Bachevalier et al, 1993) and humans (Pascalis & de Schonen, 1994) reporting stronger novelty preference with age. More importantly, the findings demonstrate for the first time the presence of a delay dependent effect that begins to emerge at 18 months, such that by this age novelty preference was weaker at the longest delays than at the shortest delays. This delay-dependent recognition memory effect remained present and became stronger as the animals matured (48 months).

One possible explanation for the emergence of a delay-dependent recognition memory effect by 18 months of age may relate to the rearing conditions used in our studies. Infant monkeys were nursery-reared and received social contact with peers and

human caregivers. Although these rearing conditions are adequate for the development of species-specific social behaviors (Sackett, Ruppenthal, & Davis, 2002), they are not as complex as the rich social environment in which monkeys usually navigate. The nursery-rearing procedures could have affected performance on the task given that rats raised with weekly exposure to an enriched environment perform better on a novel object recognition memory test (rodent version of VPC; Leal-Galicia, Castaneda-Bueno, Quiroz-Baez, & Arias, 2008) than rats raised in standard laboratory cages. However, this explanation seems unlikely when comparing novelty preference scores of the sham-operated controls of this study at 48 months with those of sham-operated adult animals that were mother-reared in a more naturalistic environment. Both groups performed similarly. Thus, rearing conditions do not seem to be the primary factor for the delay-dependent recognition memory effect observed at 18 and 48 months.

An alternative explanation would be that critical changes in the neural substrate supporting incidental recognition memory processes emerge around 18 months of age, resulting in the delay-dependent effect observed. Given the maturational sequence of the medial temporal lobe structures with the cortical areas maturing earlier than the hippocampus, the delay-dependent effect may relate to the emergence of functional interactions between the hippocampus and the medial temporal cortical areas in support of incidental recognition memory processes. If this was the case, we would expect to find novelty preference deficits after neonatal hippocampal lesions at a time when the delay-dependent effect emerges in the sham-operated animals. In addition, given that hippocampal lesions alter novelty preference only at long delays (Zola et al., 2000; Nemanic et al., 2004), we would also expect that the recognition memory deficit after

neonatal hippocampal lesions should occur at the long but not the short delays. As discussed next, these predictions were for the most part confirmed.

Recognition memory and neonatal lesion of the hippocampus

Monkeys with selective neonatal hippocampal lesions performed just as well as sham-operated controls at both 1.5 and 6 months of age, and did not show a recognition memory deficit until the longest delay of 120 s at 18 months of age. However, this deficit was only transient since by 48 months monkeys with neonatal hippocampal lesions performed again as well as the sham-operated controls at all delays, but was re-instated when stimuli were made more difficult to discriminate. The lack of group differences at all ages and delays tested, except for the long delays of 120 s, suggests that either the hippocampal formation is not necessary to support incidental memory processes during development or that other medial temporal cortical structures known to mediate familiarity/novelty judgments in adulthood (Brown and Aggleton, 2001; Murray, 2000; Nemanic et al., 2004; Yonelinas, 2002) could support incidental recognition memory processes in the absence of a functional hippocampus during development. Alternatively, the emergence of delay-dependent recognition memory performance at 18 months of age as well as the impairment observed after neonatal hippocampal lesions at the longest delays at that same age indicate that important maturational changes in the neural substrate supporting incidental recognition memory occurred around 18 months of age in monkeys.

Given the available anatomical evidence indicating that the temporal cortical areas known to support familiarity/novelty judgments seem to be functionally mature

earlier than the hippocampal formation (see for review Alvarado and Bachevalier, 2000; Zeamer et al., 2009a), it is tempting to suggest that early in development, when the hippocampal formation is not fully mature, surrounding cortical areas, such as the perirhinal cortex may be sufficient to support incidental recognition memory processes. However, between 6 and 18 months of age, as the hippocampal formation matures, it may more fully interact with the cortical areas to support this function. Finally, with further maturation of these functional interactions, the hippocampal formation may again disengage from item-specific recognition processes, which at this time could be almost entirely mediated by the cortical areas, at least under certain conditions (e.g. easily discriminable color or black/white stimuli, but not when stimuli share similar features). This interpretation is supported by our recent observation that, as compared to neonatal hippocampal lesions, neonatal perirhinal lesions altered novelty preference at 1.5, 6 and 18 months and this impairment becomes more profound with maturation and is present even at the shortest delays tested (Zeamer and Bachevalier, 2009c, see next manuscript).

Effects of stimulus-type manipulation on the magnitude of recognition memory deficit found after neonatal and adult hippocampal lesions

Because a large corpus of research has demonstrated that in many instances neonatal lesions might have a less deleterious functional effect than the same lesions in adulthood (Benton & Tranel, 2000), it is critical to compare the effects of neonatal lesions of the hippocampus to those found when the same lesions are acquired in adulthood. We recently demonstrated that the magnitude of recognition memory impairment found after hippocampal lesions in adult monkeys is dependent on several

task parameters, including length of the delays, stimulus types and duration of encoding (Zeamer and Bachevalier, 2009b). For example, for stimulus-types, hippocampal lesions altered novelty preference only when stimuli shared many common features and when delays were 60 s or longer. Thus, the magnitude of the recognition memory deficit found after neonatal hippocampal lesions as assessed with different types of pictures (color, black/white, and black/white with overlapping features) was compared to that found after adult hippocampal lesions using the same set of stimuli. For both groups, recognition memory for each stimulus type was tested at delays of 10 s, 60 s, and 120 s. Both neonatal and adult hippocampal lesions resulted in normal incidental recognition memory at all delays for color and BW stimuli, but abolished recognition memory at long delays with the BW-OF stimuli. The findings of the present study provide further support to the proposal that the hippocampal formation may be needed to support recognition memory only under conditions (long delays, stimuli with overlapping features, duration of encoding) when the medial temporal cortical areas are insufficient to support this function. Together with recent neuroimaging and electrophysiology findings (Pihlajamaki et al., 2004; Köhler, Danckert, Gati, & Menon, 2005; Danckert et al., 2007; Bakker, Kirwan, Miller, & Stark, 2008; Ramsøy, Liptrot, Skimmage, Lund, Sidaros, Christensen, et al., 2009), the present lesion data offer additional evidence that the neural processes essential to support familiarity/novelty judgments in the hippocampus may differ from those of the medial temporal cortical areas.

Nevertheless, although the present findings support earlier evidence suggesting that hippocampal damage affects recognition memory when the stimuli share many overlapping features (Ranganath, Johnson, & D'Esposito, 2000; Holdstock et al., 2002;

Pihlajamaki et al., 2004; Danckert et al., 2007; Bakker et al., 2008; Zeamer and Bachevalier, 2009b), they also indicate that this effect was more profound in animals receiving the lesions in adulthood than in those receiving their lesions early in development. Thus, both neonatal hippocampal lesions and adult lesions abolished novelty preference at the long delays for the BW-OF stimuli. However, at shorter delays, the neonatal hippocampal lesions, unlike the adult lesions, spared novelty preference. One possible explanation for the differences in the magnitude of the recognition memory deficit may relate to lesion size. It has previously been suggested that the magnitude of the recognition impairment was more profound after partial hippocampal lesions than after more complete lesions (Baxter and Murray, 2001; Bachevalier and Meunier, 1996; Mumby, Wood, Duva, Kornecook, Pinel, & Phillips, 1996). Thus, it is possible that the neonatal lesions, which resulted in a moderate deficit, were more extended than the adult lesions, which resulted in a more profound deficit. However, the hippocampal lesion extent in the three adults in the earlier study averaged 71.7% (36%, 89%, and 90%; Zeamer and Bachevalier, 2009b) and was comparable to that of the five animals of the current study averaging 56.9% (33%, 44%, 53%, 68%, and 87%). In addition, in both studies, novelty preference scores did not correlate with lesion extent. Any differences in novelty preference cannot be explained by lesion size and are most likely due to the age at which the animal received the lesion.

Thus, the magnitude of recognition memory impairment also depends on the timing of the lesions, with greater impairment after adult lesions than neonatal lesions. This moderate functional sparing after neonatal hippocampal lesions suggests that some recognition memory processes mediated by the hippocampal formation could still be

taken over by surrounding structures, such as the perirhinal cortex, in the case of neonatal lesions.

Conclusion

The present findings demonstrate that recognition memory processes are available in early infancy although the neural substrate that supports this function undergoes significant modifications as the subjects mature. Whereas the medial temporal cortical areas could mediate incidental recognition memory processes up to one year of age, the hippocampal formation begins to interact with the cortical areas around 18 months of age to support recognition memory. These findings add support to mounting evidence that suggests that the early developing brain may use different neural pathways to support the same cognitive functions in adults (Bates, 2004; Goldman and Rosvold, 1972; Webster, Ungerleider, & Bachevalier, 1995; Zeamer et al., 2009a). The data could also help resolve some debate in the literature concerning the lack of recognition memory deficit following developmental amnesia in humans. Vargha-Khadem and colleagues (de Haan, Mishkin, Baldeweg, & Vargha-Khadem, 2006) have reported that patients suffering from developmental amnesia due to early postnatal damage to the hippocampus show profound deficits in declarative memory processes but relatively normal recognition processes. Although the authors concluded that the findings supported the view that the hippocampal formation was not critical to support recognition memory, an alternative interpretation of their data would be that, due to the timing of the hippocampal damage, the recognition memory processes normally supported by the hippocampal formation could be mediated, at least in part, by the medial temporal cortical areas.

Figure Legends

Figure 1: Example of a BW VPC trial.

Subjects were familiarized to a novel image for an accumulated 30 s. Following a delay of 10 s, 30 s, 60 s, or 120 s, they were given two retention tests, in which the now familiar image was paired with a novel image, separated by a 5-s delay. Each retention test remained on the screen for 5 s after the animal initially looked. The measure of recognition memory was the animal's preference to look longer at the novel image during the retention test. Subjects were given Color trials at all ages, and BW and BW-OF trials at 48 months.

Figure 2: Lesion Reconstruction for Group Neo-Hibo

Each column depicts coronal sections through the hippocampal formation of a macaque brain. Top left column depicts in gray the intended lesion as reconstructed onto five anterior-posterior (top to bottom) levels. The numerals to the left of each coronal section indicate the distance in millimeters from the interaural plane. The remaining five columns depict the extent of lesion for each case in Group Neo-Hibo, as estimated from FLAIR MR images and reconstructed onto sections of the normal macaque infant brain. Abbreviations: A – amygdala; amts – anterior middle temporal sulcus; ERh – entorhinal cortex; ots – occipitotemporal sulcus; pmts – posterior middle temporal sulcus; PRh – perirhinal cortex; rs – rhinal sulcus; sts – superior temporal sulcus; TE, TEO and TH/TF – cytoarchitectonic fields as described by von Bonin and Bailey (1947).

Figure 3: MR images for Case Neo-Hibo-2 and Neo-C-2

Coronal MRI sections through the hippocampal formation of Case Neo-Hibo-2 (left and middle) and Case Neo-C-2 (right). The post-surgical FLAIR (left) depicts hypersignals resulting from edema caused by cell death and the 1 Year post-surgical T1 (middle) depicts the amount of hippocampal volume reduction that has resulted from the neurotoxin injection. Arrows indicate enlarged ventricles due to loss of hippocampal tissue.

Figure 4: Novelty preference scores for Group Neo-C

Mean percent of time (± SEM) spent viewing the novel Color stimuli for animals with neonatal sham operations (Group Neo-C) across each age and delay. Chance performance is depicted by the horizontal dashed line.

Figure 5: Novelty preference scores for each age and delay

Mean percent of time (± SEM) spent viewing the novel Color stimuli for animals with neonatal sham operations (Group Neo-C, open square) and animals with neonatal hippocampal lesions (Group Neo-Hibo, black diamond) across each age and delay. * indicates $p < .05$. Other conventions as in Figure 4.

Figure 6: Novelty preference scores for Color, BW and BW-OF stimuli

Mean percent of time (± SEM) spent viewing the novel stimuli across all delays for each stimulus type (A) and at each delay for the BW-OF stimuli (B). White bars in A and open squares in B are scores of animals with neonatal sham lesions. Black bars in A and

black diamonds in B are scores of animals with neonatal hippocampal lesions. Note that data for Color stimuli are those already presented on Figure 5 when subjects were tested as adults. Abbreviations: Color: color pictures of objects; BW: black and white pictures of objects; BW-OF: black and white pictures of stimuli with overlapping features. Other conventions as in Figures 4 and 5.

Figure 7: Comparison of novelty preference scores between adult and neonatal lesion groups

Mean percent of time (± SEM) spent viewing the novel stimuli across the three stimulus types (A) for animals that had received sham lesions in adulthood (Group C, cross hatched bars) and for animals that had received sham lesions in infancy (Group Neo-C, white bars). B) scores across the three delays with BW-OF stimuli for animals receiving their lesions in adulthood (dashed lines) and for animals receiving their lesions in infancy (solid lines). Scores of sham-operated controls are indicated by the open squares and those of animals with hippocampal lesions are indicated by the black diamonds. Other conventions as in Figures 4 and 5.

Figure 1

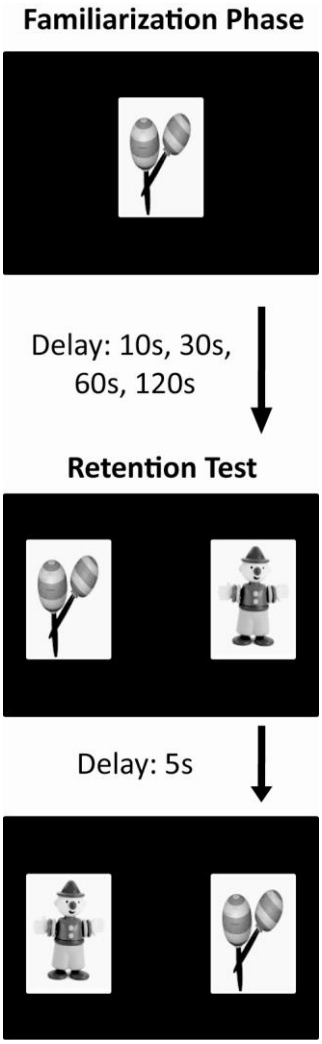
Familiarization Phase
Delay: 10s, 30s, 60s, 120s
Retention Test
Delay: 5s

Figure 2

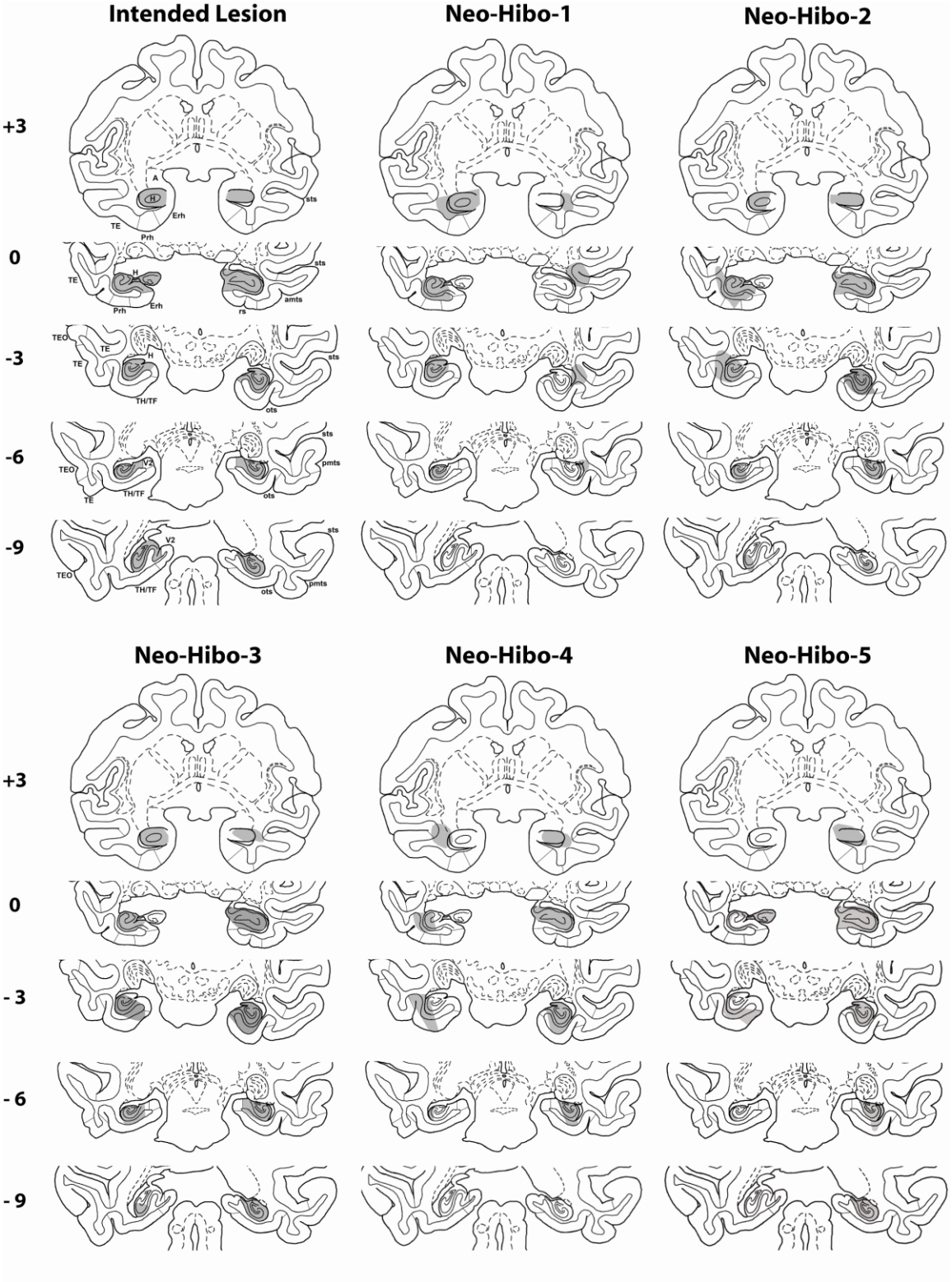
Intended Lesion
Neo-Hibo-1
Neo-Hibo-2
Neo-Hibo-3
Neo-Hibo-4
Neo-Hibo-5
+3
0
-3
-6
-9
- 3
- 6
- 9
A
H
V2
TE
Prh
Erh
sts
ots
rs
amts
pmts
TEO
TH/TF

Figure 3

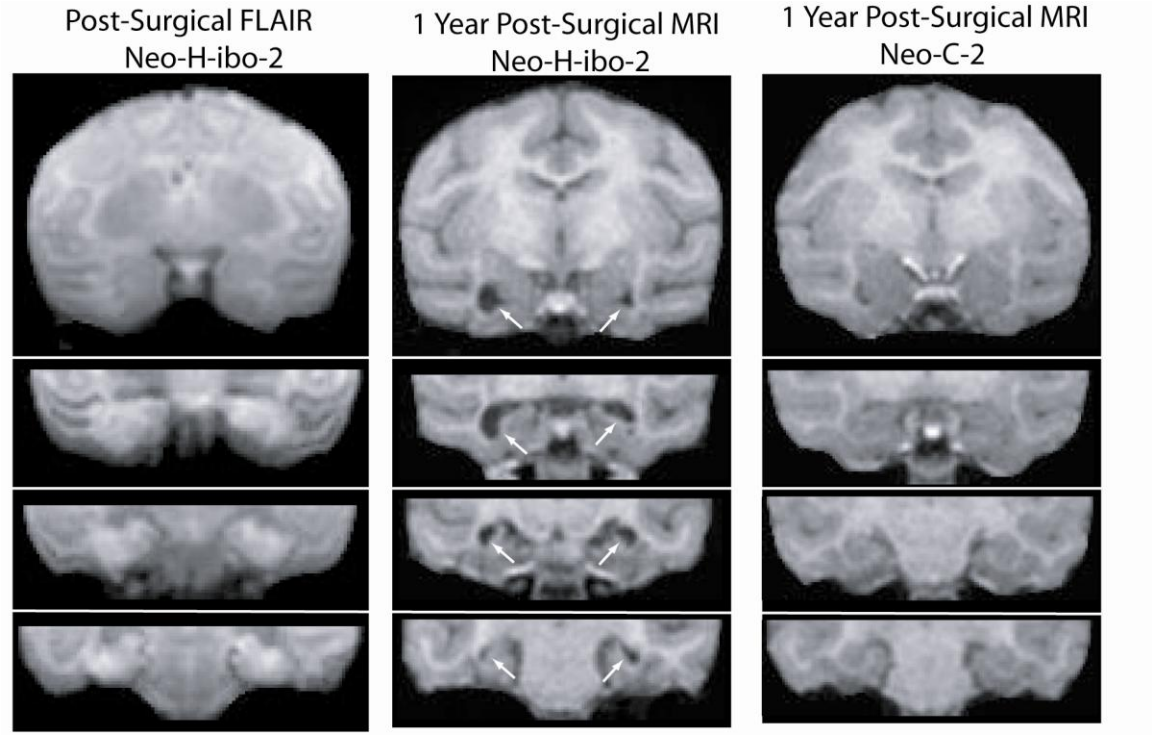
Post-Surgical FLAIR
Neo-H-ibo-2
1 Year Post-Surgical MRI
Neo-H-ibo-2
1 Year Post-Surgical MRI
Neo-C-2

Figure 4

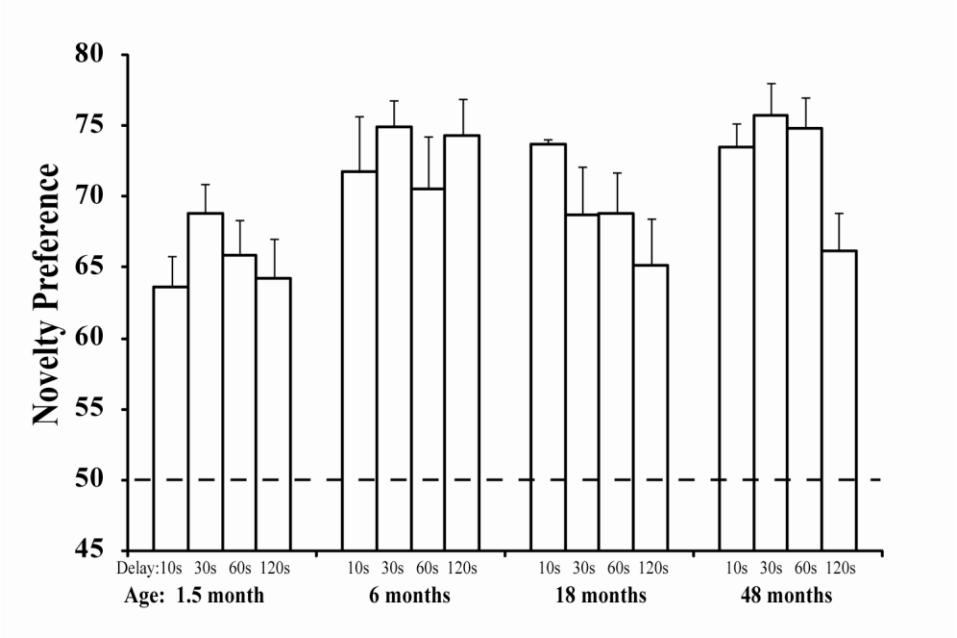
Novelty Preference
80
75
70
65
60
55
50
45
Delay:10s 30s 60s 120s
10s 30s 60s 120s
10s 30s 60s 120s
10s 30s 60s 120s
Age: 1.5 month
6 months
18 months
48 months

Figure 5

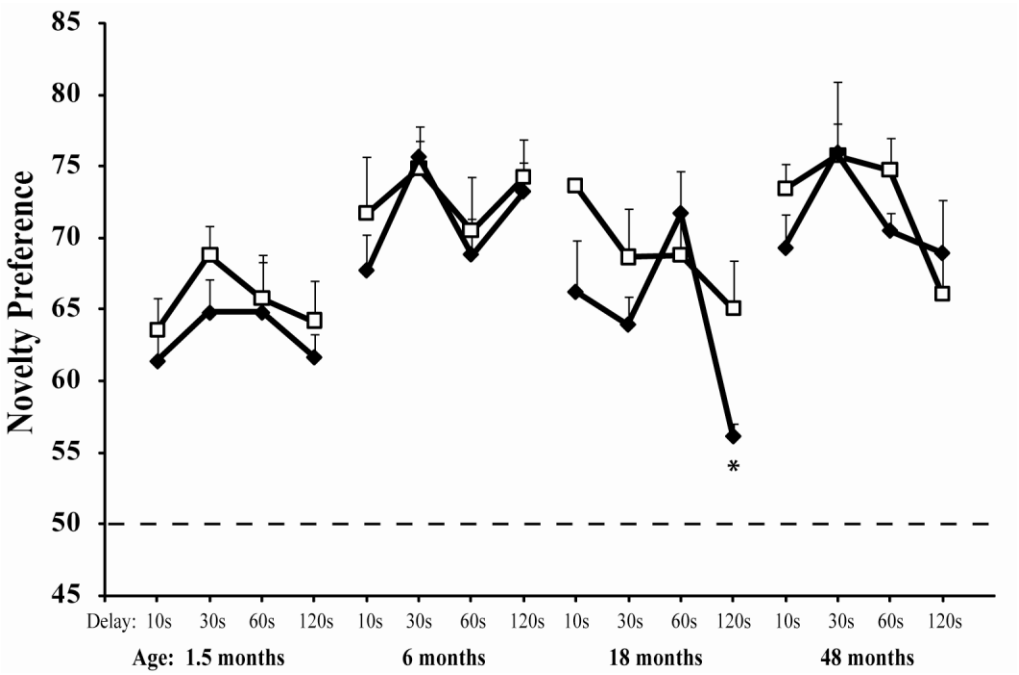
Novelty Preference
85
80
75
70
65
60
55
50
45
*
Delay: 10s 30s 60s 120s 10s 30s 60s 120s 10s 30s 60s 120s 10s 30s 60s 120s
Age: 1.5 months
6 months
18 months
48 months

Figure 6

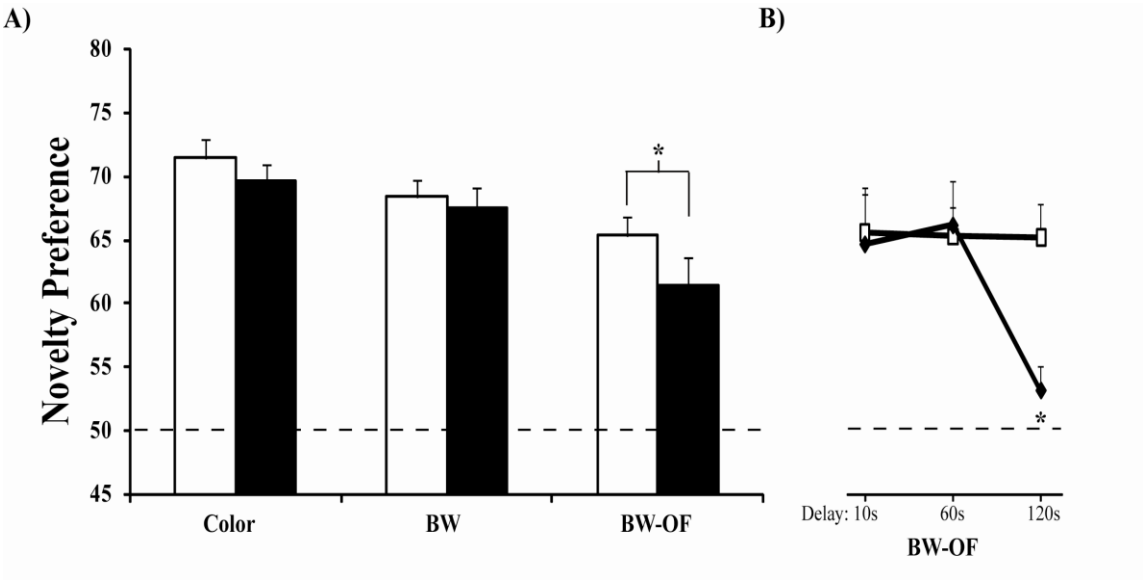
A)
B)
Novelty Preference
80
75
70
65
60
55
50
45
*
Color
BW
BW-OF
Delay: 10s
60s
120s
*
BW-OF

Figure 7

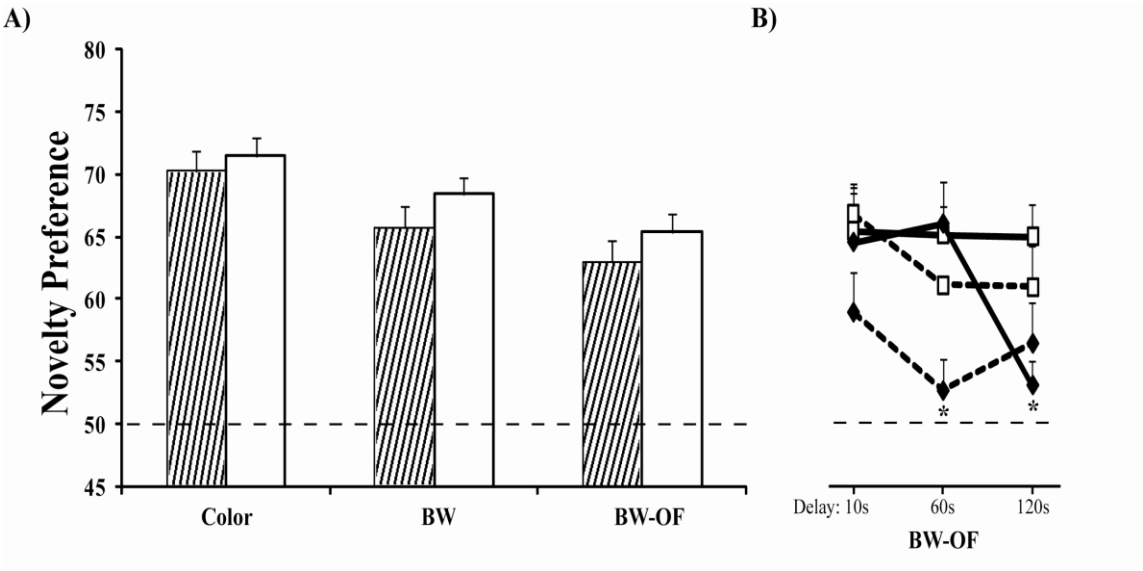
A)
B)
Novelty Preference
80
75
70
65
60
55
50
45
Color
BW
BW-OF
Delay: 10s
60s
120s
BW-OF
*
*

Table 1: Percent of intended and unintended damage

Percent damage to the hippocampal formation for the five subjects in Group Neo-Hibo as estimated from pre- and post-surgery coronal MR images: L% - percent damage to the left hemisphere; R% - percent damage to the right hemisphere; X% - average damage to both hemispheres; W% - weighted average damage to both hemispheres (W% = (L% x R%)/100); TH/ TF: cytoarchitectonic fields of the parahippocampal gyrus as defined by von Bonin and Bailey, 1947.

Cases	Intended Damage				Unintended Damage							
	Hippocampal Formation				Amygdala				TH/TF			
	L%	**R%**	**X%**	**W%**	**L%**	**R%**	**X%**	**W%**	**L%**	**R%**	**X%**	**W%**
Neo-Hibo-1	63.8	2.9	33.2	1.9	14.0	0.0	7.0	0.0	3.1	0.5	1.8	0.0
Neo-Hibo-2	54.4	80.9	67.6	44.0	0.0	0.0	0.0	0.0	21.4	2.7	12.1	0.6
Neo-Hibo-3	78.5	96.3	87.4	75.6	1.7	0.0	0.8	0.0	6.1	5.5	5.8	0.3
Neo-Hibo-4	20.3	67.3	43.8	13.7	0.0	4.7	2.4	0.0	15.3	0.0	7.6	0.0
Neo-Hibo-5	20.7	84.0	52.6	17.4	0.0	4.9	2.4	0.0	6.1	4.0	5.1	0.2
Avg	**47.5**	**66.3**	**56.9**	**30.5**	**3.1**	**1.9**	**2.5**	**0.0**	**10.4**	**2.5**	**6.5**	**0.2**

Table 2: Percent volume reduction measured at 1 year of age

Percent of hippocampal formation volume reduction and sparing for the five subjects in Group Neo-H-ibo as estimated from 1 year post-surgical coronal MR images: L% - percent damage to the left hemisphere; R% - percent damage to the right hemisphere; X% - average damage to both hemispheres.

	% Volume Reduction		
Cases	**L%**	**R%**	**X%**
Neo-Hibo-1	27.6	10.7	19.1
Neo-Hibo-2	61.2	72.9	67.0
Neo-Hibo-3	54.7	47.8	51.3
Neo-Hibo-4	33.6	61.7	47.6
Neo-Hibo-5	49.2	64.0	56.6
Avg	45.3	51.4	48.3

Table 3: Individual novelty preference scores for all Color trials for each subject

Avg – average; SE – standard error of the mean

Novelty Preference (%)

Age	**1.5 months**				**6 months**				**18 months**				**48 months**			
Delay	**10s**	**30s**	**60s**	**120s**	**10s**	**30s**	**60s**	**120s**	**10s**	**30s**	**60s**	**120s**	**10s**	**30s**	**60s**	**120s**
Neo-C-1	68.2	77.4	60.7	63.9	82.8	79.1	83.0	79.1	72.6	79.5	77.5	79.2	74.9	71.0	81.2	72.0
Neo-C-2	71.5	64.0	62.4	66.8	81.4	76.4	75.6	79.9	73.5	74.4	74.0	68.1	79.5	76.8	68.1	76.4
Neo-C-3	56.9	65.5	73.2	54.1	67.4	80.8	69.9	72.9	74.3	70.5	69.2	62.0	71.0	77.1	77.5	64.5
Neo-C-4	63.0	65.7	59.6	62.5	61.7	69.8	67.1	75.6	75.6	69.2	71.9	65.0	71.0	69.7	73.9	63.3
Neo-C-5	63.1	67.7	73.4	75.0	76.3	69.6	71.9	76.1	73.3	56.1	61.0	54.5	67.8	85.2	79.7	61.1
Neo-C-6	58.8	72.6	65.6	63.1	60.9	73.8	55.9	62.1	72.6	62.5	59.6	61.8	76.5	74.8	68.3	60.0
Avg	***63.6***	***68.8***	***65.8***	***64.2***	***71.7***	***74.9***	***70.6***	***74.3***	***73.7***	***68.7***	***68.9***	***65.1***	***73.5***	***75.8***	***74.8***	***66.2***
SE	***2.2***	***2.1***	***2.5***	***2.8***	***4.0***	***1.9***	***3.7***	***2.7***	***0.5***	***3.4***	***2.9***	***3.4***	***1.8***	***2.3***	***2.3***	***2.7***
Neo-Hibo-1	64.0	61.5	78.3	63.1	75.7	71.9	63.1	76.3	60.2	57.5	73.6	58.1	*63.3*	*70.1*	*69.7*	*67.4*
Neo-Hibo-2	59.3	70.7	63.9	56.1	71.2	74.8	68.7	76.6	78.0	67.2	76.6	58.1	*67.6*	*82.9*	*71.3*	*72.0*
Neo-Hibo-3	67.7	70.1	57.5	63.9	65.9	77.7	73.6	76.8	62.9	68.9	69.2	55.4	*73.9*	*62.5*	*71.3*	*63.1*
Neo-Hibo-4	56.1	59.7	68.0	65.4	63.7	71.1	75.4	69.3	71.1	63.2	77.8	55.6	*66.5*	*90.9*	*66.5*	*60.7*
Neo-Hibo-5	59.9	62.1	56.5	59.9	62.2	82.8	63.5	67.2	59.0	63.1	61.4	53.7	*75.4*	*73.5*	*73.8*	*81.7*
Avg	***61.4***	***64.8***	***64.8***	***61.7***	***67.7***	***75.7***	***68.9***	***73.3***	***66.3***	***64.0***	***71.8***	***56.2***	***69.3***	***76.0***	***70.5***	***69.0***
SE	***2.0***	***2.3***	***4.0***	***1.7***	***2.5***	***2.1***	***2.5***	***2.1***	***3.6***	***2.0***	***3.0***	***0.9***	***2.3***	***5.0***	***1.2***	***3.7***

Table 4: Individual novelty preference scores for all BW and BW-OF trials for each subject

Avg – average; SE – standard error of the mean

Novelty Preference (%)						
Stimulus	**BW**			**BW-OF**		
Delay	**10s**	**60s**	**120s**	**10s**	**60s**	**120s**
Neo-C-1	69.0	71.8	60.6	67.7	69.4	71.5
Neo-C-2	67.3	74.7	71.1	71.7	66.4	66.5
Neo-C-3	70.1	66.3	66.0	71.4	73.4	73.8
Neo-C-4	66.4	75.4	65.0	69.2	63.0	59.1
Neo-C-5	74.7	74.1	53.8	65.2	57.6	58.7
Neo-C-6	66.1	72.0	67.2	48.6	62.1	61.5
Avg	***68.9***	***72.4***	***63.9***	***65.6***	***65.3***	***65.2***
SE	***1.3***	***1.4***	***2.5***	***3.6***	***2.3***	***2.6***
Neo-Hibo-1	75.5	66.9	58.8	67.6	54.6	60.0
Neo-Hibo-2	69.8	71.5	58.7	55.9	64.3	51.3
Neo-Hibo-3	72.3	82.3	65.0	75.1	75.8	54.9
Neo-Hibo-4	66.0	67.5	57.2	55.2	67.3	49.2
Neo-Hibo-5	66.3	69.8	65.6	69.9	69.1	50.4
Avg	***70.0***	***71.6***	***61.0***	***64.7***	***66.2***	***53.2***
SE	***1.8***	***2.8***	***1.8***	***4.0***	***3.5***	***2.0***

The development of object recognition memory in rhesus macaques with neonatal lesions of the perirhinal cortex

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Abstract

Incidental recognition memory, as measured by preference for novelty in the visual paired comparison task (VPC), has been show to be present as early as 1.5 months of age and delays as long as 120 s in infant rhesus macaques. In addition, recognition memory became stronger throughout development, increasing from 1 to 6 months of age, and remaining strong through 48 months, but was delay dependent at 18 and 48 months. Monkeys with neonatal lesions of the hippocampus showed a similar pattern of novelty preference at 1, 6 and 48 months. However, at 18 months, novelty preference was significantly weaker in monkeys with neonatal hippocampal damage than in control animals at the longest delay of 120 s, although performance remained greater than chance. These data suggested that the hippocampus may not be necessary to support incidental memory processes early in development, but that this form of recognition memory may be mediated by the medial temporal cortical areas that are crucial for familiarity judgments and object memory in adults. To investigate this proposal, monkeys (*Macaca mulatta*) with neonatal perirhinal lesions (Group PRh) and sham-operated controls (Group C) were tested at 1.5, 6 and 18 months of age on the VPC task with color stimuli and intermixed delays of 10s, 30s, 60s, and 120s. Monkeys with neonatal lesions of the perirhinal cortex showed a similar increase in novelty preference between 1.5 and 6 months of age, although at these two ages performance remained significantly poorer than that of control animals. Nevertheless, with further maturation, performance deteriorated in animals with neonatal perirhinal lesions as compared to that of controls. In contrast to findings from monkeys with lesions of the perirhinal cortex acquired in adulthood, novelty preference was above chance at all ages and delays. Thus,

early incidental recognition memory processes can be supported by the perirhinal cortex, but, because the impairment was less in magnitude than that seen in adults, the data suggest that other temporal cortical areas may support incidental recognition memory processes in the absence of a functional perirhinal cortex early in development.

Introduction

The role of the perirhinal cortex in recognition memory has received growing support from studies in several species including rodents, monkeys and humans (for review see Winters, Saksida, & Bussey, 2008). In monkeys, selective lesions of the perirhinal cortex either alone, or in conjunction with the entorhinal cortex or parahippocampal cortex severely impair performance on object recognition memory tasks, including delayed nonmatching-to-sample and delayed matching to sample (Buffalo et al., 1999; Buffalo, Ramus, Squire, & Zola, 2000; Hadfield, Baxter, & Murray, 2003; Nemanic et al., 2004; Gaffan & Murray, 1992, Meunier et al., 1993; Baxter & Murray, 2001; Zola-Morgan et al., 1989) as well as the visual paired comparison (VPC) task (Nemanic et al., 2004). In the VPC task, the recognition deficit emerges at very short delays of only a few seconds and contrasts with the recognition memory impairment seen only at delays longer than 60 s after selective hippocampal lesions. Similarly, in humans, damage to the medial temporal lobe, which included the perirhinal cortex, impaired performance on a yes/no recognition memory task at delays greater than 6 seconds, as compared to the impairment seen at longer delays (25 s) in patients with damage limited to the hippocampal formation (Buffalo et al., 1998). In addition, damage to the temporal lobe that included the perirhinal cortex but spared the hippocampus, impaired the ability of human subjects to make familiarity judgments in the remember/know paradigm (Bowles et al., 2007). Finally, neuroimaging studies in humans (Pihlajamaki et al., 2004; Danckert et al., 2007; Ramsøy et al., 2009) have shown that the perirhinal cortex (PRh) plays a role in processing and encoding novel object information. While the contribution of the perirhinal cortex to recognition memory

processes in adults is well established, its contribution to the early developing recognition memory abilities that have been demonstrated in both humans (Fagan, 1970; Diamond, 1995; Pascalis & de Schonen, 1994) and monkeys (Gunderson & Sackett, 1984; Bachevalier et al., 1993; Zeamer et al., 2009b) remained to be tested.

In a recent longitudinal study, we examined the development of object recognition memory in monkeys using the visual paired comparison (VPC) task, a task known to measure incidental recognition memory processes (Zeamer et al., 2009b). Infant monkeys were tested on the VPC task at four time points during development (1.5, 6, 18 and 48 months) using delays varying from 10 to 120 s and pictures of color stimuli. At the youngest age of 1.5 months, infant monkeys showed novelty preference at all delays and this preference became more robust by 6 months of age. However, at 18 months of age, even when novelty preference remained well above chance level, a delay-dependent effect emerged, such that novelty preference was stronger at the shortest than at the longest delays. In addition, monkeys with neonatal neurotoxic hippocampal lesions performed as well as controls at 1.5 and 6 months of age, and, by 18 months of age, also showed a delay-dependent effect. However, the decrease in novelty preference across the delays was steeper in animals with neonatal hippocampal lesions than in control animals. Thus, at the longest delay of 120 s, novelty preference of animals with neonatal hippocampal lesions was significantly weaker than that of controls. This pattern of findings suggested that, in the absence of a functional hippocampus, the intact recognition memory seen at the earlier ages of 1.5 and 6 months in the operated monkeys could be supported by the medial temporal cortical areas, and more specifically the

perirhinal cortex, that have been shown to be crucial for familiarity judgments and object recognition memory in adults.

To test this hypothesis, in the present study, we assessed the magnitude of recognition memory impairment following neonatal neurotoxic perirhinal lesions, using the same recognition task (VPC), delays (10 to 120 s), color stimuli and age points (1.5 to 18 months) as in our previous study. We predicted that if the perirhinal cortex mediates recognition memory processes measured with the VPC task at an early age, animals with neonatal perirhinal lesions should be impaired on the task at all ages and with short as well as long delays. Preliminary reports of the findings were published in abstract form (Zeamer & Bachevalier, 2009a).

Methods

Subjects

The subjects were eight full-term infant rhesus monkeys (*Macaca mulatta*), four males and four females born from multiparous females at the Yerkes National Primate Research Center (YNPRC) breeding colony (Atlanta, GA). Between 1-2 days of age, newborn infants were brought from the YNPRC breeding colony (Atlanta, GA) to the primate nursery at the YNPRC Main Station. Between 10 and 12 days postnatally, two animals underwent a sham operation (Group Neo-C) and the remaining six received stereotaxic guided ibotenic acid lesions of the perirhinal cortex (Group Neo-PRh). To allow comparisons with the animals that had received neonatal hippocampal lesions or sham-operations and that were prepared earlier at the University of Texas-Houston, these new groups of monkeys were reared using similar procedures by experimenters that had

raised the monkeys of our earlier study (Zeamer et al., 2009b). All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Texas Houston, where this study began, and Emory University, and were kept constant between the two institutions. All animals received similar rearing procedures, including social interactions with age-matched peers and human caregivers as described previously (see Goursaud and Bachevalier, 2007).

Surgical Procedures

Magnetic Resonance Imaging (MRI) scans and Determination of Injection Coordinates

All animals received MRI scans immediately prior to surgery. The scans for Group Neo-PRh were used to determine the precise stereotaxic coordinated for injection of ibotenic acid (Nemanic et al., 2000). The subjects were anesthetized with Ketamine Hydrochloride and Xylazine (10 mg/kg of 7:3 Ketamine Hydrochloride, 100mg/ml, and Xylazine, 20mg/ml, i.m.), and intubated to inhale isofluorane gas (1.0-3.0%, v/v, to effect) to maintain adequate anesthesia throughout the procedure. After being placed in the stereotaxic apparatus, the brain was imaged with a GE Signa 1.5 Tesla Echo Speed scanner (GE Medical Systems, Milwaukee, WI at UT-Houston) and with a 3 T Siemens Magnetom Trio system (Siemens Medical Solutions, Malvern, PA at YNPRC). Two sets of coronal images were taken; the first set was used to calculate the neurotoxin injection sites in the perirhinal cortex (3D T1-weighted fast spoiled gradient (FSPGR)-echo sequence, TE = 2.6 ms, TR = 10.2 ms, 25° flip angle, contiguous 1 mm sections, 12 cm FOV, 256 x 256 matrix). The second set, used to measure edema caused by cell death from the neurotoxin, (Fluid Attenuated Inversion Recovery (FLAIR) sequence, TE = 140 ms, TR = 10000 ms, inversion time (TI) = 2200ms, contiguous 3 mm sections, 12 cm

FOV, 256 x 256 matrix) were acquired in three series, offset by 1mm posteriorly. Approximately one week after surgery, as well as 1 year after surgery, animals in Group Neo-PRh underwent a second series of scans in order to evaluate lesion extent using methods previously described by Nemanic and colleagues (2002).

Surgery

All surgical procedures were performed under deep anesthesia using aseptic techniques. The animal was maintained on Isofluorane gas (1.0 – 2.0%, v/v, to effect) throughout surgery, and an IV drip containing dextrose and 0.45% sodium chloride was used to maintain normal hydration. To prevent hypothermia, a heating pad was placed underneath the animal, and the veterinary staff monitored all vital signs until the animal fully recovered from anesthesia.

Once the scalp was shaved and the skin disinfected with Nolvasan, a long lasting local anesthetic (Marcaine 25%, 1.5 ml) was injected along the incision line. An incision was made longitudinally along the midline of the skin, and the galea was then retracted. Using an electric drill, craniotomies (1 cm wide x 2.5 cm long) were made bilaterally and Bone wax (Ethicon, Inc., Somerville, NJ; 2.5g size) was used to prevent excessive bleeding. The dura was then opened wide enough to allow a Hamilton syringe, held by Kopf electrode manipulators (David Kopf Instruments, Tujunga, CA), to be lowered simultaneously into each hemisphere. Three injection sites were chosen at 2mm intervals along the length of the perirhinal cortex, bilaterally, and 0.4µl ibotenic acid (Biosearch Technologies, Novato, CA, 10 mg/ml in PBS, pH 7.4) was injected at each site at a rate of 0.4 µl per minute. The dura, galea and skin were then sutured and the animal was removed from isoflurane and kept under constant supervision until fully recovered.

Starting 12 hours prior to surgery and lasting 7 days after, subjects received dexamethazone sodium phosphate (0.4 mg/kg, i.m.) to reduce edema and Cephazolin (25 mg/kg, i.m.) to prevent infection. For pain relief, acetaminophen (10 mg/kg, p.o.) was given four times a day for 3 days following surgery.

Sham operated controls underwent the same procedures, however once the dura was cut, no needle was lowered.

Post-surgical MRI scans and MRI-based lesion evaluation

No histological evaluations were available because all animals are still currently participating on other cognitive experiments at this time. Previously described procedures to measure the extent or damage were used (Málková et al., 2001; Nemanic et al., 2002). Areas of hypersignal, representing the edema caused by cell death, were visually identified on the one-week post-surgical FLAIR images and then plotted onto matching coronal drawings of a normal monkey brain. Image J® was used to measure the surface area (in pixels^2) of damage to the left and right perirhinal cortex, as well as unintended damage to adjacent structures. The percentage of volume damage was calculated by dividing the volume of damage to the perirhinal cortex by the volume of that structure in a normal monkey. The percent reduction of the perirhinal cortex at 1 year of age was measured from T1 scans taken at 1 year of age using the same procedures.

Behavioral Apparatus:

Subjects were held by an experimenter at 1.5 and 6 months of age, and were seated in a custom plexiglass primate chair at 18 months. The subjects viewed stimuli on a 19" display monitor from approximately 40 cm away. The experimenter controlled the presentation of the stimuli on a Dell laptop connected to the monitor. A video camera

(Sony Digital8 TRV-140) was mounted on the top of the monitor such that the eyes of the animal were clearly visible on a TV screen next to the experiment to monitor the animal's looking behavior at all times. The camera output was fed through a time-date generator and connected to a JVC VCR so that the eye movements were recorded for later coding. To reduce outside noise, a whit noise generator was used.

Pre-Training:

Infants tested at 1.5 months of age were first habituated to the testing room by watching Disney cartoons for three days prior to testing, for an increasing period of time each day (15, 20 and 25 minutes). At 6 months of age, they received only one 20-minute habituation day before testing began. At 18 months, animals were first trained to enter the primate chair, which took from 2-5 days per animal. Once they were acclimated to the chair, they received one 20-minute habitation day in the testing room. Subjects did not remain in the testing room for more than 45 minutes a day at any age tested. Subjects were neither food nor water deprived during testing, and occasionally received a treat during the inter-trial interval on test day.

VPC task:

All monkeys were tested at 1.5, 6 and 18 months on the visual paired comparison task (VPC), which takes advantage of the subject's natural tendency to look at the novel object without earning a reward. Stimuli were color pictures of variegated objects from multiple categories, chosen to be as different as possible, varying in color, shape and texture, selected from a pool of 800,000 clipart images (Nova Art Explosion 800,000 Clip Art). Each trial consisted of a Familiarization Phase and two Retention Tests (Figure 1). In the Familiarization Phase, animals fixated on a novel stimulus in the center of the

screen until they reached 30s of cumulative looking time. This was followed by a variable delay of 10s, 30s, 60s or 120s, and then two Retention Tests, separated by a 5s delay, in which the now familiar image was paired with a novel image. Each retention test stayed on the screen for 5s after the animal initially looked. Also, the left/right position of the two images was reversed during the Retention Tests, and the left/right position of the novel image varied pseudo-randomly on the first Retention Test. Each trial was separated by a 30s inter-trial interval (ITI). The screen remained black for each delay period and each ITI. Animals received 3-8 trials per day and received treats only at 18 months, and only during an ITI. They received 10 trials at each delay at each age, for a total of 120 trials per subject. The measure of recognition memory was the animal's preference to look longer at the novel image during the Retention Tests.

Data Analysis

For each trial, eye movements during each retention test were analyzed frame-by-frame to calculate the amount of time the animal spent looking towards the familiar and the novel stimuli. Two observers independently scored the trials (inter-observer reliability: pearson $r = 0.931$). Each observer was blind to the initial right/left position of the novel image, as well as whether the subject was in Group Neo-C or Group Neo-PRh. From this, the total amount of time the animal spent looking towards the novel image was calculated [(time looking at novel / total time looking) X 100)]. In addition, the Total Familiarization Phase, which is the length of time it takes the animal to accumulate 30s of looking behavior, and the Total Retention Time, which is the total amount of time the animal looks during the two retention tests, were measured for each trial. If an animal had

a Total Retention Time of less than 1 second, the trial was excluded, which only occurred 7 times out of 1680 trials.

Statistical Analysis

We first compared performance of animals of Group Neo-C (N = 6) prepared at UT-Houston with the two sham-operated monkeys added at the YNPRC, to assess whether any small changes in our experimental procedures affected performance of the animals on the VPC task. Given the comparable performance of these two groups of sham-operated monkeys, we combined them into a single sham-operated group (Group Neo-C). Thus, statistical comparisons will be made between Group Neo-C (N = 8), Group PRh (N = 6) and Group Neo-H (N = 6).

All statistical analyses were carried out using the SPSS 17.0 software, using a General Linear Model ANOVA with a between subject comparison for the effect of Group (Neo-C, Neo-PRh, and Neo-H) and within subject comparisons using repeated measures for the effects of Age (1.5, 6 and 18 months) and Delay (10, 30, 60 and 120s). When sphericity was not assumed, a Huynh-Feldt correction was used. Post-Hoc Tukey tests were conducted when group differences reached significance, and one-sample t-tests were used to evaluate group differences from chance. Additionally, planned comparisons were performed between the control group and the experimental group, using a one-sided Planned Comparison (Pedhazur, 1982). These statistical analyses were carried out on three parameters of the VPC task, i.e. the Total Familiarization Phase and the Total Retention Time, which measure viewing behaviors and novelty preference, which measures recognition memory.

Results

Evaluation of perirhinal cortex lesion:

All cases received extensive bilateral PRh lesions, varying from 67.06% to 83.34% (average: 73.6%; Table 1). In addition, all cases had unintended bilateral damage to the lateral entorhinal cortex (ERh), ranging from 5.42% to 34.49% (average: 20.57) and to area TE, ranging from 0.10% to 7.11% (average: 2.15%). Three cases, Neo-PRh-1, -2, and -6 had a small amount of damage to the amygdala (average: 2.48%) and four cases, Neo-PRh-1, -3, -5 and -6 had a minute amount of damage to the anterior hippocampus (average: 0.81%). Figure 2 shows the extent of hypersignals seen on the post-surgical FLAIR for each case, plotted on matched coronal sections through a normal infant brain. A representative MR FLAIR image (Case Neo-PRh-3) is seen in Figure 3.

Comparison between control animals

Comparisons between sham-operated control subjects raised at UT-Houston (cases Neo-C-1 to -6) and those raised at YNPRC (Cases Neo-C-7 and -8) revealed no Group differences or interactions between Group and Delay or Group and Age in Total Familiarization Phase (Group: $F(1,6) = 3.59$, $p > .1$; Group X Age: $F(2,12) = .98$, $p > .4$; Group X Delay: $F(3,18) = .01$, $p > .9$), Total Retention Time (Group: $F(1,6) = 1.62$, $p > .2$; Group X Age: $F(2,12) = 2.6$, $p > .1$; Group X Delay: $F(3,18) = 1.06$, $p > .3$) or novelty preference scores (Group: $F(1,6) = .034$, $p > .8$; Group X Age: $F(2,12) = .62$, $p > .5$; Group X Delay: $F(3,18) = .26$, $p > .8$; Table 2). Because there were no differences in

looking behavior based on whether the animal was reared at UT-Houston or YNPRC, the eight subjects were combined to form Group Neo-C for all remaining statistics.

Effect of neonatal perirhinal cortex lesion:

Comparisons between animals with neonatal perirhinal lesions and the sham-operated controls revealed no effects of group or delay on Total Familiarization Time or Total Retention Time. However, the effect of age was significant for Total Familiarization Time ($F(2,24) = 5.62$, $p < 0.01$) with both groups taking less time to accumulate the 30-s viewing to the stimulus at 1.5 and 6 months of age (.51 and 1.88 min, respectively) than at 18 months (2.59 min; $ps < .0001$), and for Total Retention Time ($F_{HUYNH\text{-}FELDT}(1.66,19.94) = 8.75$, $p < 0.01$) with both groups looking slightly longer at the two stimuli during the retention tests at 1.5 and 6 months of age (7.36 and 7.75 s, respectively) than at 18 months (6.26 s; $ps < .0001$).

Group differences emerged for novelty preference ($F(1, 12) = 11.32$, $p < .01$), and the Group X Age interaction was also significant ($F(2, 24) = 3.46$, $p < .05$). As shown in Figure 4, this interaction indicated that across all delays animals with neonatal perirhinal lesions obtained novelty preference scores lower than control animals at the three ages ($p = 0.06$ at 1.5 months; $p < 0.01$ at 6 and 18 months). Despite this group difference, similarly to control animals, novelty preference in animals with neonatal perirhinal lesions became more robust from 1.5 months to 6 months ($ps < .01$ for both). However, although control animals maintained robust novelty preference at 18 months, those with neonatal perirhinal lesions obtained novelty preference scores significantly lower at 18 months than at 6 months ($p < .001$). Thus, by 18 months of age, although novelty

preference in Group Neo-PRh remained above chance level at all delays, it was also significantly weaker than that of Group Neo-C at all delays ($p < .01$ at 10 s; $p = .06$ at 30s, and $p < .01$ at 60 and 120 s, see Figure 5).

Comparison with animals with neonatal hippocampal lesions:

The data from animals with neonatal lesions of the hippocampus tested under the same conditions, and used here for comparison to animals with neonatal PRh lesions, have previously been published by Zeamer and colleagues (2009b). There was an overall effect of age ($F(2, 18) = 20.51$, $p < 0.001$) on novelty preference scores, with both groups showing stronger novelty preference from 1 to 6 months of age ($p < .05$). In addition, there was a marginal Group effect ($F(1,9) = 3.87$, $p = .08$) and Group by Delay interaction ($F(3,27) = 2.633$, $p = .07$). Further analysis within each age revealed that Group Neo-PRh had significantly weaker novelty preference than Group Neo-Hibo at 6 and 18 months ($p < 0.05$ for both; Figure 6). Furthermore, Group Neo-PRh showed weaker novelty preference with 30-s and 120-s delays at 6 months ($p = .08$ at 30s; $p = .07$ at 120s) and with the 60-s delay at 18 months ($p < .01$). Importantly, while Group Neo-Hibo differed from Group Neo-C only at the longest delay of 120 s at 18 months, Group Neo-PRh differed at all delays at 18 months.

Discussion

The primary goal of this study was to examine whether the perirhinal cortex supports early developing recognition memory processes, as measured by VPC, and to compare the effects of neonatal perirhinal versus neonatal hippocampal lesions on the

development of incidental recognition memory. Neonatal lesions of the perirhinal cortex impacted recognition memory at the earliest age of 1.5 months and this impairment became more profound with maturation, although novelty preference was not totally abolished at any ages or any delays. In addition, neonatal perirhinal lesions resulted in a recognition memory deficit that occurred at younger ages and at shorter delays than that found after neonatal hippocampal lesions.

Recognition memory and neonatal perirhinal cortex lesion

Comparisons between the two sham-operated control subjects raised with Group Neo-PRh and the six sham-operated control subjects raised with Group Neo-H revealed no differences in any of the parameters measured. Thus, any differences between the control group and animals with perirhinal lesions cannot simply be attributed to small variations in rearing conditions.

Neonatal lesions of the perirhinal cortex resulted in significant decreases in novelty preference that became more profound with maturation, but performance remained above chance at all ages and delays. Thus, animals with neonatal perirhinal lesions showed weaker novelty preference relative to sham-operated controls as early as 1.5 months of age. Although their performance increased from 1.5 to 6 months of age as it did in the controls, they still performed significantly more poorly at 6 months. In addition, at 18 months of age, unlike controls in whom novelty preference remained stable, novelty preference in animals with perirhinal lesions declined and was weaker than that of sham-operated controls at every delay tested. These novel findings suggest that the perirhinal cortex is critical to support incidental memory processes early in

development. These data are also consistent with adult electrophysiological (Xiang & Brown, 1998; Brown & Xiang, 1998), imaging (Pihlajamaki et al., 2004; Kohler et al., 2005; Buffalo et al., 2006; Lee et al., 2006; Danckert et al., 2007; Ramsøy et al., 2009), and molecular activation (Zhu et al., 1995; 1996; 1997, Wan et al., 1999; Davachi and Goldman-Rakic, 2001) studies that have shown that the perirhinal cortex is involved in object processing and memory.

Although the data suggest a role for the perirhinal cortex in the development of incidental recognition memory, the effects observed may also be due to partial unintended damage to the entorhinal cortex (range: 5.4 to 34.5%, Table 1), which receives information about object perceptual characteristics from the perirhinal cortex (for review see Eichenbaum et al., 2007). However, there were no correlations between subjects' novelty preference scores and their amount of entorhinal cortex damage. In addition, in the adult monkeys, lesions of the entorhinal cortex yielded recognition memory deficits that were milder than lesions of the perirhinal cortex alone or in combination with the entorhinal cortex (Eacott, Gaffan, & Murray, 1994; Leonard, Amaral, Squire, & Zola-Morgan, 1995; Meunier et al., 1993). Therefore, although the contribution of the entorhinal cortex to the deficit in recognition memory cannot be entirely ruled out, it seems at the present time unlikely.

Despite the recognition memory deficit, novelty preference in animals with neonatal perirhinal lesions was not totally abolished, a finding that contrasts with the lack of novelty preference found in monkeys with adult lesions of the perirhinal cortex at delays as short as 10s (Nemanic et al., 2004, Buffalo et al., 1999). One possible explanation for this difference in the magnitude of recognition impairment between

neonatal and adult lesions of the perirhinal cortex could be related to differences in the extent of the perirhinal lesion. However, the extent of perirhinal lesions in the adult animals of the two previous studies averaged 87% (Nemanic et al., 2004) and 74% (Buffalo et al., 1999), and was comparable to that found in animals with neonatal perirhinal lesions (average: 74%). In addition, there were no correlations between VPC performance and lesion size, suggesting that the weaker performance exhibited by monkeys with neonatal lesions of the perirhinal cortex cannot simply be attributed to a difference in lesion size.

Yet another possibility is that the monkeys in the present study have not reached maturity, and given the progressive deficit found between 6 months and 18 months, it is possible that with further maturation, more profound deficits in recognition memory will emerge. Furthermore, the differences in novelty preference scores between the neonatal and adult lesions could be due to the type of stimuli used. It has previously been shown that the magnitude of novelty preference in normal monkeys varied according to the type of stimuli used, with stronger novelty preference with easily discriminable color pictures than with black and white pictures of objects that share many features (Zeamer and Bachevalier, 2009b). As compared to the color stimuli used in the current studies, the two studies investigating the effects of perirhinal lesions acquired in adulthood used black and white stimuli that were back projected onto a screen, which could have resulted in poorer quality images. In addition, the perirhinal cortex is known to play a role in object discrimination, mainly when the objects share many overlapping features (Barense, Bussey, Lee, Rogers, Davies, Saksida, et al., 2005; Buckley, Booth, Rolls, & Gaffan,, 2001; Bartko, Winters, Cowell, Saksida, & Bussey, 2007; Bussey, Saksida, & Murray,

2002; 2003; 2006). Therefore, it is possible that the difference in novelty preference scores between animals with neonatal lesions and those with adult lesions of the perirhinal cortex could be due to the type of stimuli. However, because perirhinal lesions in rodents severely impaired their performance on NOP even when three dimensional objects were used (Ennaceur, Neave, & Aggleton, 1996; Bussey, Duck, Muir, & Aggleton, 2000; Winters et al., 2004), the difference between the outcomes of the neonatal and adult perirhinal lesions may be related to the timing of the lesions. Although further studies are needed to re-test the animals when they will reach adulthood (48 months) and using different types of stimuli, the data currently suggest that, although PRh supports incidental recognition memory processes in infancy, the neural substrate mediating these processes appears more widespread in early infancy than in adulthood.

Comparison with neonatal hippocampal lesion

As with the comparison to sham-operated controls, animals with neonatal perirhinal cortex lesions showed weaker preference for novelty than animals with neonatal hippocampal lesions at both 6 and 18 months of age when tested with the same stimuli and under the same testing conditions. Because there were no differences in rearing or testing conditions between the two groups, all differences in preference for novelty can be attributed to the specific structures involved in the neonatal lesions. There were two important differences between the two types of lesions. First, the neonatal perirhinal lesions affected performance as early as 1.5 months of age indicating that at this earlier age the perirhinal cortex as well as other medial temporal cortical areas may support incidental recognition memory processes. Second, although the neonatal

perirhinal cortex lesions altered performance even at the short delays, the neonatal hippocampal lesions impacted recognition memory only at the longest delay. This finding provides further support to a growing consensus that of all the medial temporal lobe structures, the perirhinal cortex is critical for the support of object recognition memory as measured by all recognition tasks used so far, such as the VPC, NOP, DNMS and DMS (Winters et al., 2008). On the other hand, while several studies have shown that the hippocampus plays a delay-dependent role, there are just as many that have found no deficit after hippocampal lesion. Even when a deficit is reported, it is not as severe as the deficit in animals with lesions of the perirhinal cortex (Prusky Douglas, Nelson, Shabanpoor, & Sutherland, 2004; Nemanic et al., 2004). The current findings further support such a distinction between the contribution of these two structures to recognition memory processes. Additional support for this idea comes from electrophysiological findings which show that neurons in the perirhinal cortex preferentially respond to novel over familiar stimuli (for review see Winters et al., 2008), supporting a role for the perirhinal cortex in making familiarity judgments (Brown and Aggleton, 2001; Eichenbaum, 2007).

Figure Legends

Figure 1: Example of a VPC trial.

Subjects were familiarized to a novel image for an accumulated 30 s. Following a delay of 10 s, 30 s, 60 s, or 120 s, they were given two retention tests, in which the now familiar image was paired with a novel image, separated by a 5-s delay. Each retention test remained on the screen for 5 s after the animal initially looked. The measure of recognition memory was the animal's preference to look longer at the novel image during the retention test. Subjects were given Color trials at all ages, and BW and BW-OF trials at 48 months.

Figure 2: Lesion Reconstruction for Group Neo-PRh

Each column depicts coronal sections through the perirhinal cortex of a macaque brain. Left column depicts in gray the intended lesion as reconstructed onto four anterior-posterior (top to bottom) levels. The numerals to the left of each coronal section indicate the distance in millimeters from the interaural plane. The remaining six columns depict the extent of lesion for each case in Group Neo-PRh, as estimated from 1 week post-surgical FLAIR MR images and reconstructed onto sections of the normal macaque infant brain. Abbreviations: A – amygdala; ERh – entorhinal cortex; H – hippocampus; PRh – perirhinal cortex; rs – rhinal sulcus; sts – superior temporal sulcus; TE – cytoarchitectonic field as described by von Bonin and Bailey (1947).

Figure 3: MR images for Case Neo-PRh-3

Coronal pre-surgical T1 MR images (left column) and post-surgical FLAIR MR images (right column) through the perirhinal cortex of Case Neo-PRh-3. The white areas on the FLAIR images depict hypersignals resulting from edema caused by cell death.

Figure 4: Novelty preference scores for each age

Mean percent of time (± SEM) spent viewing the novel stimuli for animals with neonatal sham operations (Group Neo-C, white bars) and animals with neonatal perirhinal cortex lesions (Group Neo-PRh, black bars) across each age. Chance performance is depicted by the horizontal dashed line. * indicates $p < .05$; ** indicates $p < .01$; † indicates $p < 0.01$.

Figure 5: Novelty preference scores for each age and delay

Mean percent of time (± SEM) spent viewing the novel stimuli for animals with neonatal sham operations (Group Neo-C, open square) and animals with neonatal perirhinal cortex lesions (Group Neo-PRh, black circle) across each age and delay. Chance performance is depicted by the horizontal dashed line. Other conventions as in Figure 4.

Figure 6: Comparison between Group Neo-PRh and Group Neo-Hibo

Mean percent of time (± SEM) spent viewing the novel stimuli for animals with neonatal sham-operations (Group Neo-C, open square), neonatal perirhinal cortex lesions (Group Neo-PRh, black circle) and animals with neonatal hippocampal lesions (Group Neo-H-ibo, black diamond) across each age and delay. Chance performance is depicted by the horizontal dashed line. Other conventions as in Figure 4.

Figure 1

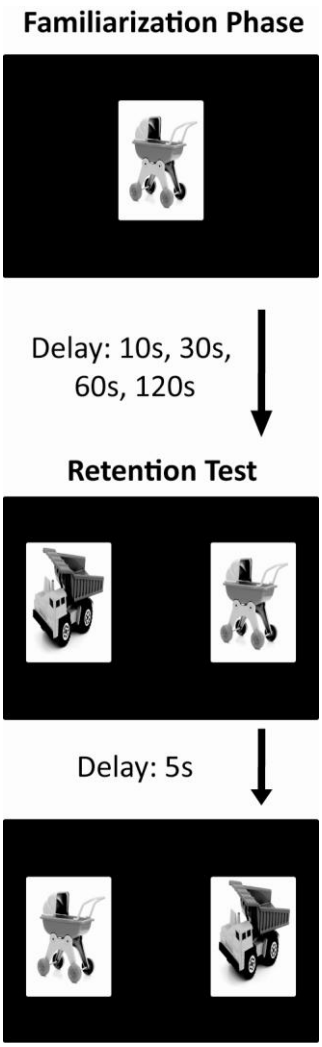
Familiarization Phase
Delay: 10s, 30s, 60s, 120s
Retention Test
Delay: 5s

Figure 2

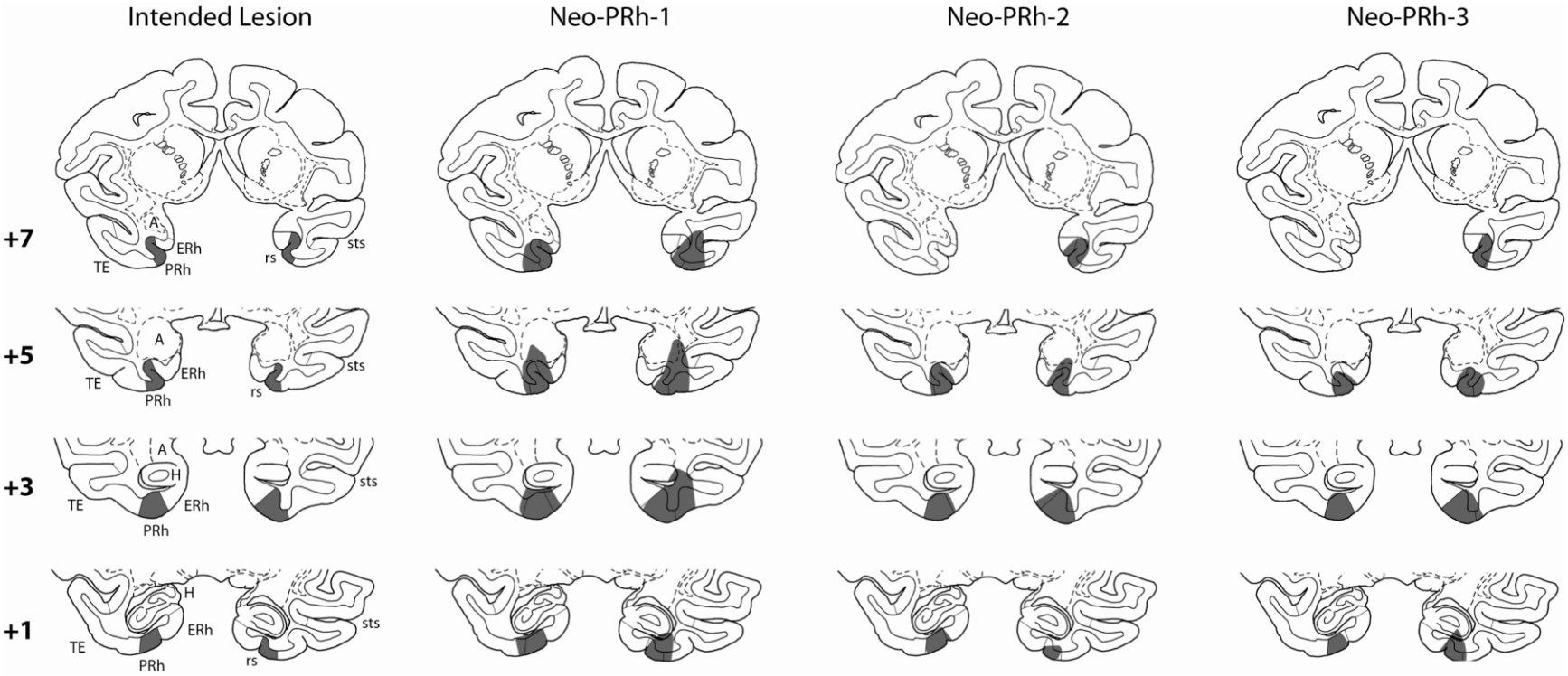
Intended Lesion
Neo-PRh-1
Neo-PRh-2
Neo-PRh-3
+7
+5
+3
+1
A
H
ERh
PRh
TE
rs
sts

Figure 2 continued

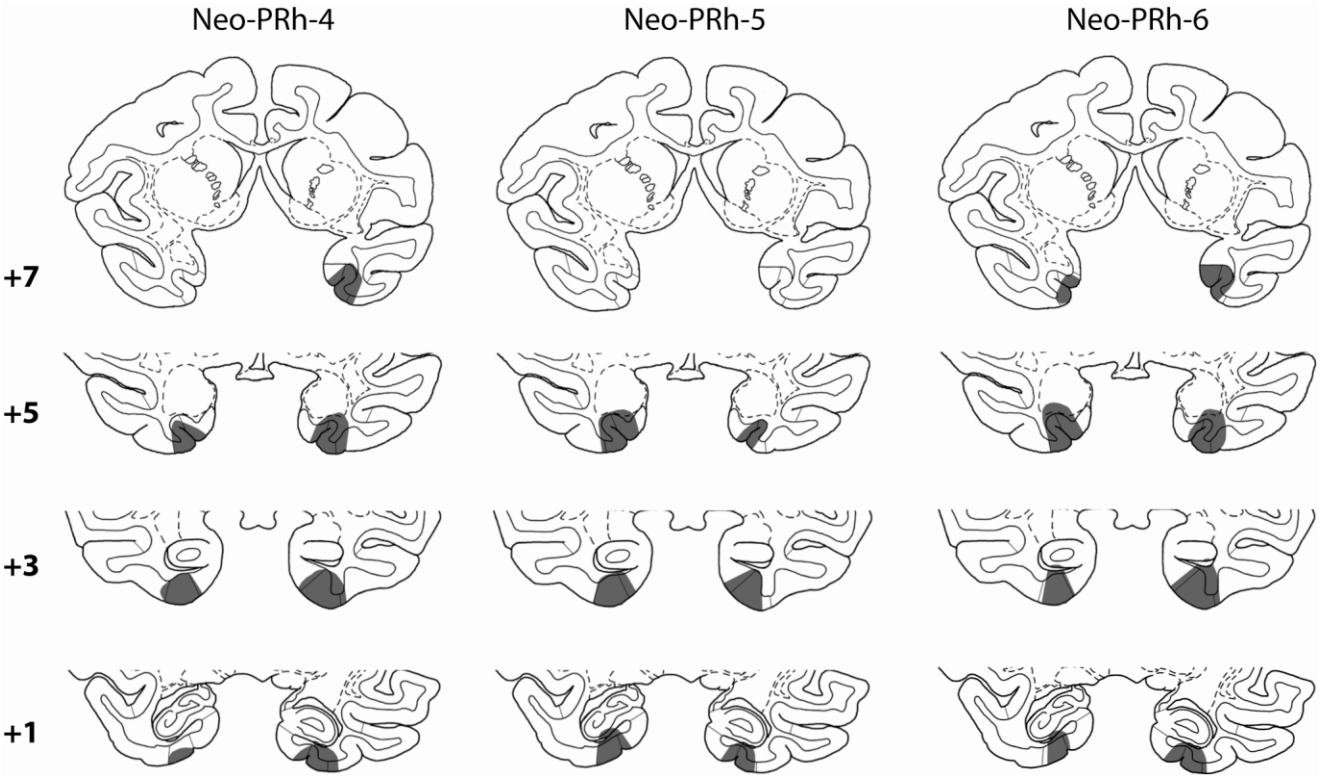

Figure 3

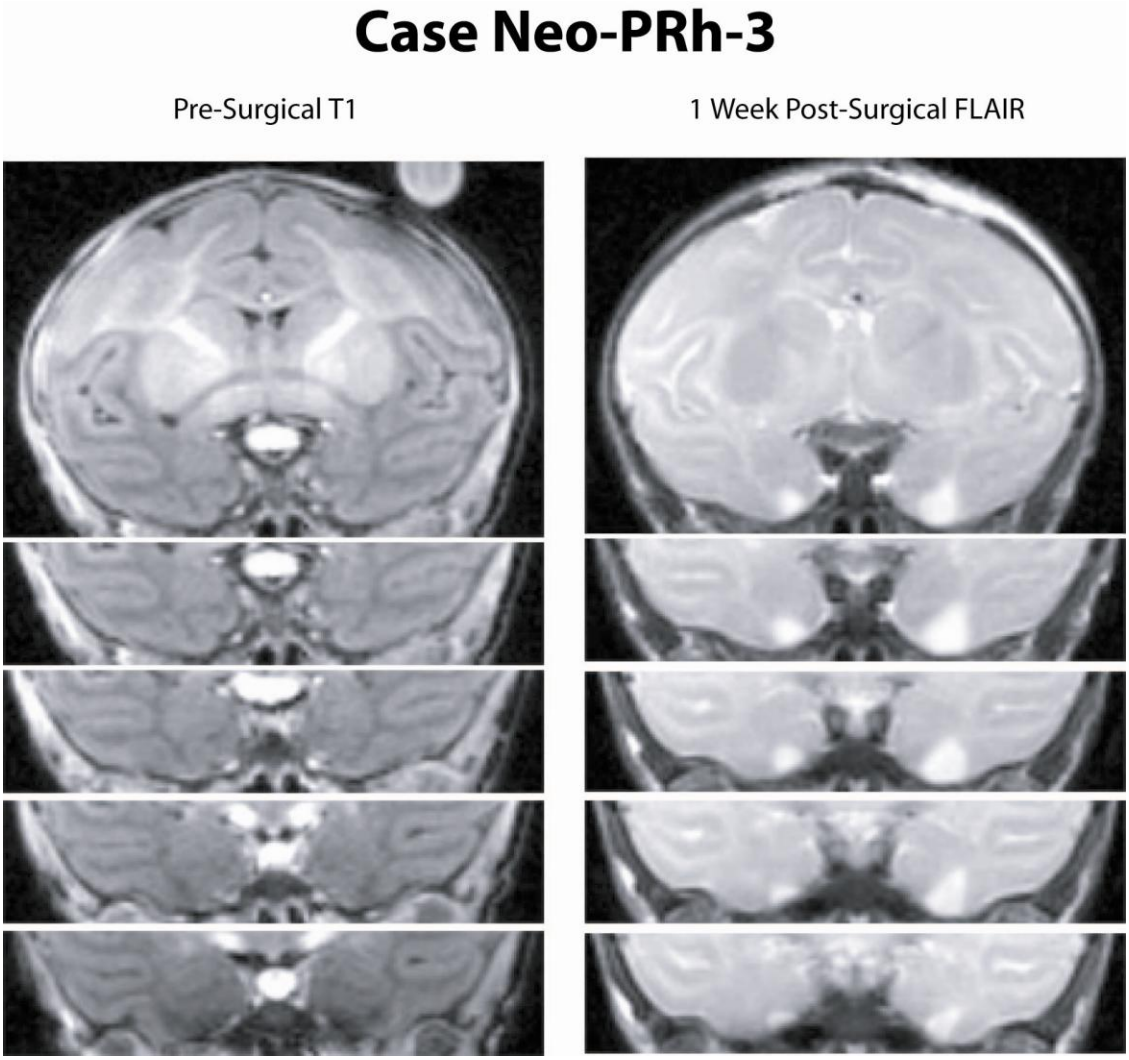

Figure 4

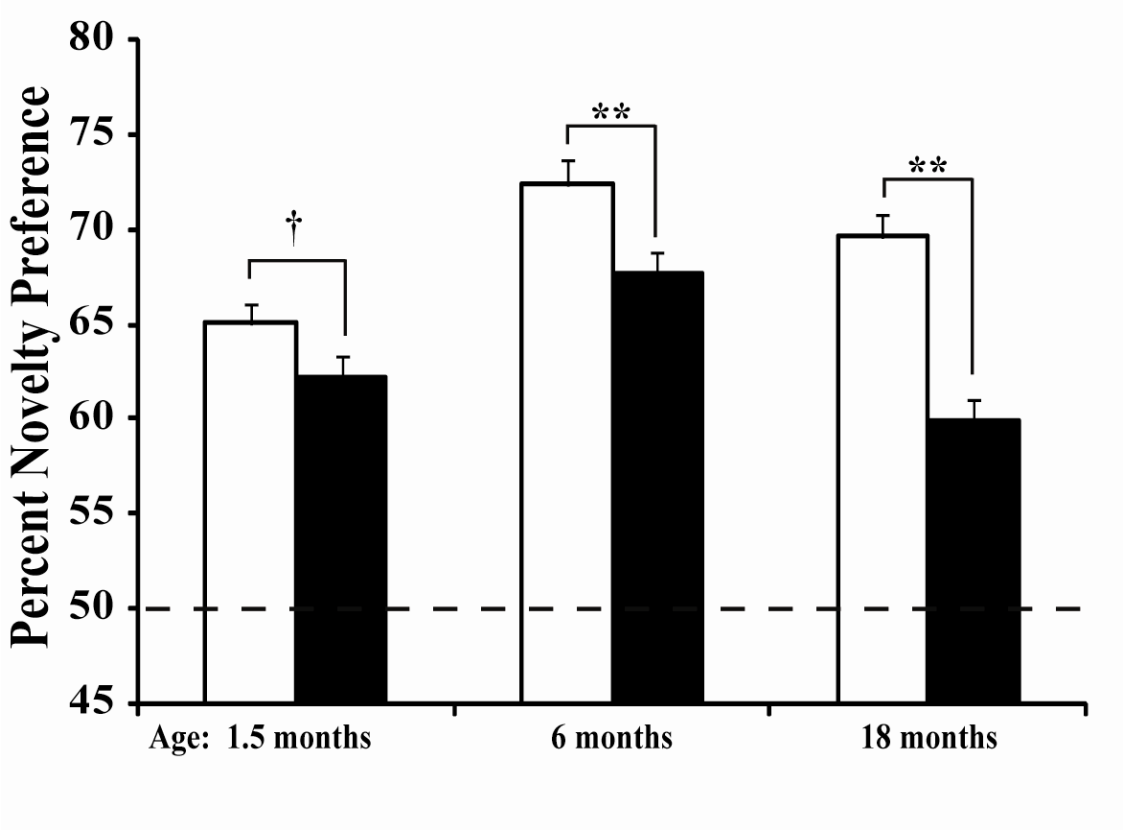
Percent Novelty Preference
80
75
70
65
60
55
50
45
†
**
**
Age: 1.5 months
6 months
18 months

Figure 5

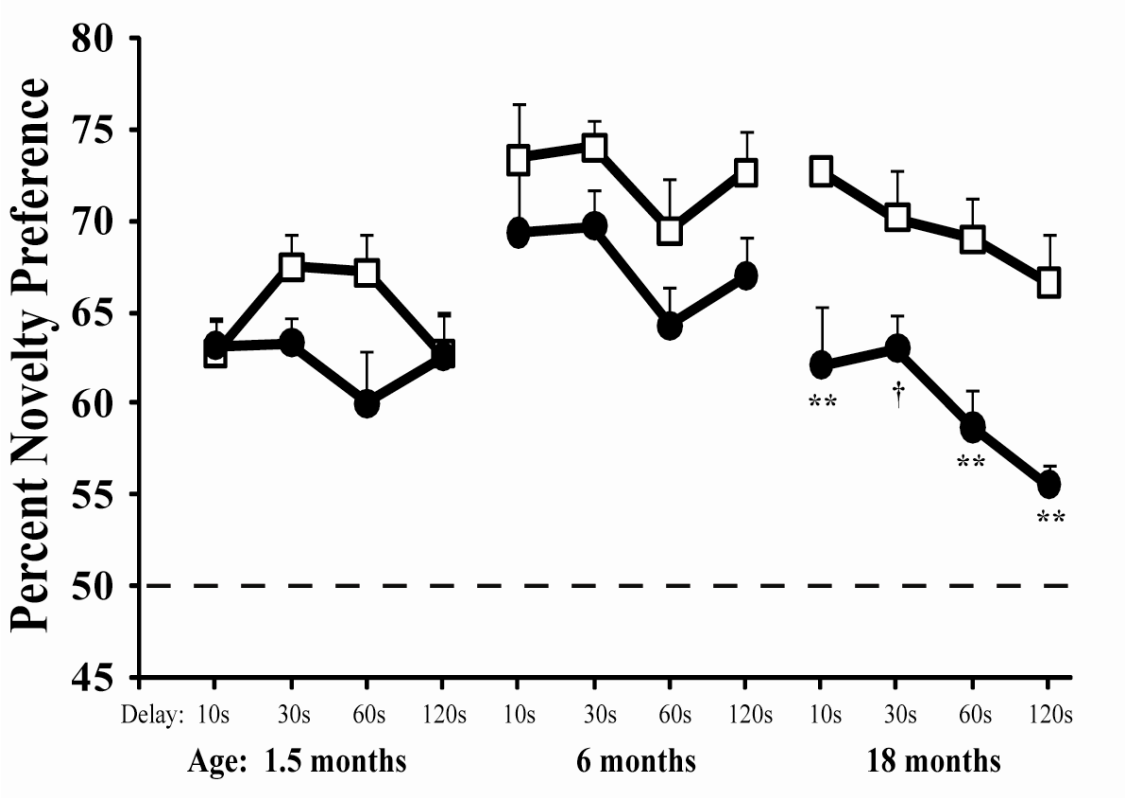
Percent Novelty Preference
80
75
70
65
60
55
50
45
Delay: 10s 30s 60s 120s 10s 30s 60s 120s 10s 30s 60s 120s
Age: 1.5 months
6 months
18 months
**
†
**
**

Figure 6

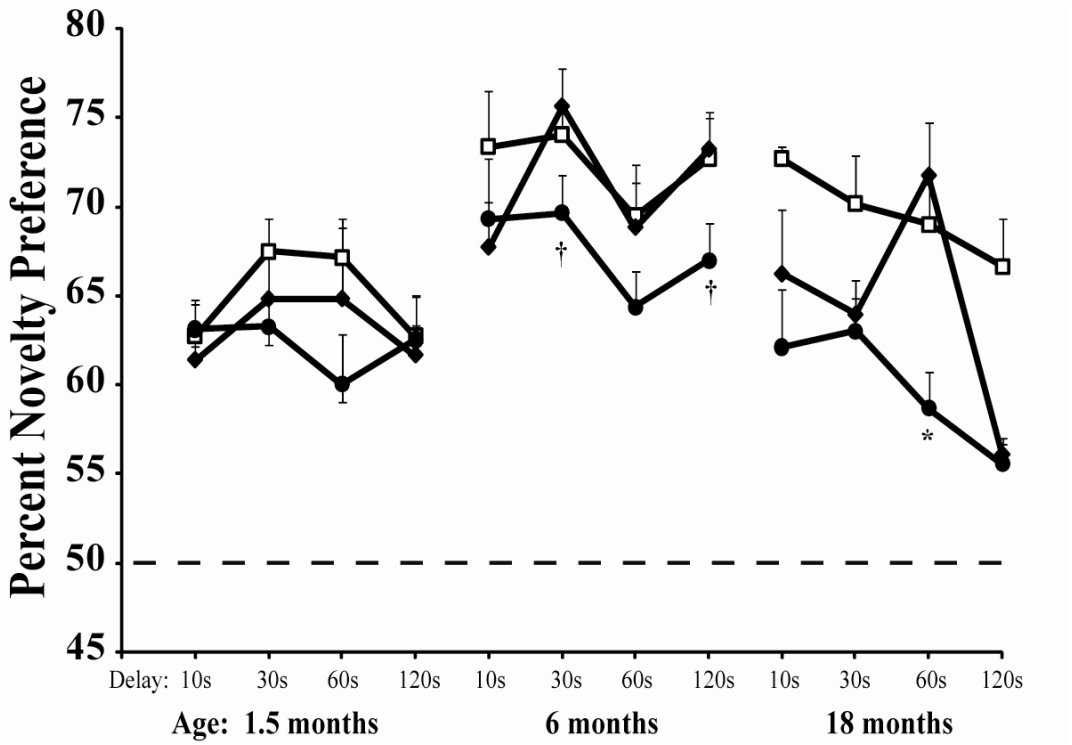
Percent Novelty Preference
80
75
70
65
60
55
50
45
Delay: 10s 30s 60s 120s 10s 30s 60s 120s 10s 30s 60s 120s
Age: 1.5 months
6 months
18 months
†
†
*

Table 5: Percent of intended and unintended damage

Percent damage to the perirhinal cortex for the six subjects in Group Neo-PRh as estimated from pre- and post-surgery coronal MR FLAIR images: L% - percent damage to the left hemisphere; R% - percent damage to the right hemisphere; X% - average damage to both hemispheres; W% - weighted average damage to both hemispheres (W% = (L% x R%)/100); Avg – average for the entire group; ERh – entorhinal cortex.

Cases	Intended Damage PRh				Unintended Damage Erh			
	L%	**R%**	**X%**	**W%**	**L%**	**R%**	**X%**	**W%**
Neo-PRh-1	89.8	76.9	83.3	69.0	28.5	2.3	15.4	0.6
Neo-PRh-2	68.2	70.6	69.4	48.1	17.7	20.7	19.2	3.7
Neo-PRh-3	65.4	81.0	73.2	53.0	7.7	3.1	5.4	0.2
Neo-PRh-4	59.4	74.7	67.1	44.4	11.5	17.8	14.7	2.1
Neo-PRh-5	75.9	66.8	71.4	50.7	38.6	29.9	34.2	11.5
Neo-PRh-6	74.1	80.3	77.2	59.5	25.3	43.6	34.5	11.1
Avg	*72.1*	*75.1*	*73.6*	*54.1*	*21.6*	*19.6*	*20.6*	*4.9*

Table 6: Individual novelty preference scores for all trials for each subject

Avg – average; SE – standard error of the mean.

Novelty Preference (%)

Age	**1.5 months**				**6 months**				**18 months**			
Delay	**10s**	**30s**	**60s**	**120s**	**10s**	**30s**	**60s**	**120s**	**10s**	**30s**	**60s**	**120s**
Neo-C-1	68.2	77.4	60.7	63.9	82.8	79.1	83.0	79.1	72.6	79.5	77.5	79.2
Neo-C-2	71.5	64.0	62.4	66.8	81.4	76.4	75.6	79.9	73.5	74.4	74.0	68.1
Neo-C-3	56.9	65.5	73.2	54.1	67.4	80.8	69.9	72.9	74.3	70.5	69.2	62.0
Neo-C-4	63.0	65.7	59.6	62.5	61.7	69.8	67.1	75.6	75.6	69.2	71.9	65.0
Neo-C-5	63.1	67.7	73.4	75.0	76.3	69.6	71.9	76.1	73.3	56.1	61.0	54.5
Neo-C-6	58.8	72.6	65.6	63.1	60.9	73.8	55.9	62.1	72.6	62.5	59.6	61.8
Neo-C-7	62.6	60.6	68.1	57.6	80.8	70.3	62.6	65.2	69.2	73.2	73.3	69.7
Neo-C-8	57.9	66.6	74.6	59.2	76.0	72.9	70.2	70.7	70.7	76.1	66.0	73.0
Avg	***62.8***	***67.5***	***67.2***	***62.8***	***73.4***	***74.1***	***69.5***	***72.7***	***72.7***	***70.2***	***69.0***	***66.7***
SE	***1.8***	***1.8***	***2.1***	***2.2***	***3.1***	***1.5***	***2.9***	***2.3***	***0.7***	***2.7***	***2.3***	***2.7***
Neo-PRh-1	62.5	65.0	60.6	59.8	70.8	66.9	68.1	63.5	73.2	70.9	58.2	60.0
Neo-PRh-2	66.8	60.2	46.8	64.0	59.5	65.0	59.2	59.1	51.7	58.7	55.1	54.0
Neo-PRh-3	56.8	59.0	64.5	55.9	83.6	77.1	63.1	69.2	68.8	61.0	59.7	52.9
Neo-PRh-4	60.6	64.1	60.7	62.9	67.1	68.8	63.4	66.2	57.5	59.6	54.1	53.9
Neo-PRh-5	66.6	68.7	61.5	59.9	71.3	75.1	72.3	70.7	64.0	63.6	68.1	55.8
Neo-PRh-6	65.7	62.9	66.2	73.0	63.9	65.2	60.3	73.4	57.7	64.5	57.2	57.1
Avg	***63.2***	***63.3***	***60.1***	***62.6***	***69.4***	***69.7***	***64.4***	***67.0***	***62.2***	***63.0***	***58.7***	***55.6***
SE	***1.6***	***1.4***	***2.8***	***2.4***	***3.4***	***2.1***	***2.0***	***2.1***	***3.3***	***1.8***	***2.1***	***1.1***

Discussion

The primary goal of this project was to investigate the normal development of recognition memory as measured by VPC in non-human primates, and the contribution of the hippocampus and perirhinal cortex in the development of these memory processes. Several interesting new findings emerged from the data. In normally developing monkeys, incidental recognition memory abilities emerge by 1.5 months of age, becomes stronger by 6 months of age, and go through profound changes around 18 months of age. This pattern of changes across development may in fact reflect some critical changes in the maturation of the neural substrate mediating incidental recognition memory processes. First, the presence of recognition memory early in infancy could be supported, at least in part, by the perirhinal cortex early in development. Second, the improvement in novelty preference with further maturation appears to be associated with additional refinement in the neural architecture of the sensory cortices and their interactions with the perirhinal cortex. Finally, the drastic changes in novelty preference across delay emerging around 18 months of age appear to be associated with the coming on-line of the hippocampus. At this age, the hippocampus begins to competitively interact with the perirhinal cortex and other medial temporal cortical areas to support incidental recognition memory abilities. Finally, the neonatal hippocampal lesions and even more so the neonatal lesions of the perirhinal cortex yielded some sparing of incidental recognition memory abilities in that the magnitude of the impairment observed was less than that seen in monkeys with the same lesion in adulthood. This sparing of function suggests greater plasticity between medial temporal structures to support incidental recognition memory processes.

Development of recognition memory processes.

The presence of incidental recognition memory in early infancy is consistent with previous findings in humans and non-human primates demonstrating that object recognition memory as measured by VPC is present early in infancy (Fagan, 1970; Gunderson and Sackett, 1984; Bachevalier et al, 1993; Pascalis & de Schoenen, 1994, Diamond, 1995). In addition, the present findings, as those reported in the previous studies, indicate that novelty preference becomes stronger as subjects mature. By contrast to developmental studies in humans that have generally demonstrated that recognition memory becomes more robust over long delays with maturation (Rose et al., 2004), the present findings indicated a stable level of performance across all delays tested even at the youngest age of 1.5 months. It is possible that for the monkeys this delay effect could have emerged if delays longer than 120 s had been used, if infants younger than 1.5 months of age would have been tested, and/or if shorter familiarization times would have been used. Nevertheless, an important finding of the present study that has never been shown is the occurrence of a delay-dependent effect that emerged around 18 months of age. All of these maturational changes in recognition memory processes may in fact be associated with important changes in cognitive processes that are required for recognition memory of objects to occur.

It has been fairly well established that explicit memory occurs through three stages of processing, namely encoding, consolidation and retrieval, each of which are carried out by different structures within the brain (for review see Bauer, 2004; Tulving, 1974). Encoding, which is defined as the formation of object representation and

acquisition of information, mostly involves the cortical association areas and is linked to perceptual and attentional processes. Consolidation, defined as the process by which newly acquired information is placed in longer term storage, involves the medial temporal lobe structures. Finally, retrieval, defined as the process that brings stored memories back into consciousness, involves the prefrontal cortex. Thus, explicit memory necessitates an interaction between all of these structures to create a memory of an event and to later retrieve it. Incidental recognition memory, which also measures a form of explicit memory (Nemanic et al., 2004; Manns et al., 2000; Nelson, 1997), is dependent on encoding and consolidation processes, but not on conscious retrieval processes. Thus, any changes in these cognitive processes during maturation could be responsible for the changes in recognition memory over age.

Developmental changes in encoding processes.

The improvement in novelty preference early in infancy has been thought to reflect changes in encoding processes, since younger subjects tend to require longer familiarization time and less complex stimuli than older subjects to demonstrate recognition memory in the VPC task (see for review Rose et al., 2004; Hayne, 2004). Changes in encoding processes can be associated with profound changes in sensory perception and attention. Thus, modifications of perceptual processes with age are likely supported by important changes in sensory cortical areas in early infancy. For example, for visual processing areas in monkeys, myelination of fibers in the occipital cortex is present at birth but remains mostly absent for the fibers in the visual temporal areas until 6 months of age (Payne et al., 2009, in press). Further, metabolic activity within the

ventral visual cortical areas processing object features reached adult-like levels around 4 months of age (Distler et al., 1996) and neuronal responsiveness of inferior temporal cortex to visual stimulation is immature up to 4 months of age in monkeys (Rodman et al., 1991). Finally, projections from one visual cortical area to the others are much more widespread in younger subjects and terminal cortical fields become narrower with further maturation (Webster et al., 1991). These findings suggest important architectural and functional changes within the ventral visual pathways up to 4 months of age that could explain better performance on the novelty preference task with maturation. Similarly, changes in attentional processes during infancy may explain the improvement of novelty preference with age. Thus, children with short fixation times and with increased numbers of shifts between stimuli tend to show better recognition memory than those that have longer fixation times and less shifts (Rose et al., 2001). Rose and colleagues (2004) suggested that fast lookers may be processing the information quicker, thus processing speed may also be a factor in the increased performance between 1.5 and 6 months of age. However, much less is known on the maturation of the neural substrate supporting attentional processes.

Developmental changes in consolidation processes

A general tenet in human developmental recognition memory research is that older infants remember after longer delays than younger infants (Hayne, 2004; Rose et al., 2004; Richmond & Nelson, 2007), indicating important changes in consolidation processes during maturation. Yet, the current data are not consistent with the human literature. Thus, at both 1.5 and 6 months, novelty preference remained stable across all

delays tested. As discussed above, this difference may be due to several factors, such as the youngest age tested, the length of the delay, and/or the length of encoding time used. Overall, there is little systematic work on developmental changes in consolidation processes and the trade-offs between age, type of stimuli, and familiarization time (Rose et al., 2004).

Nevertheless, despite this stable level of novelty preference across delays at the youngest ages, a delay-dependent effect emerged around 18 months and remained present at 48 months, such that at these two ages novelty preference was weaker at the long delay of 120-s than at the short delays. The emergence of a delay-dependent effect suggests that important changes in consolidation and/or incidental retrieval processes occur around this age and these changes could in turn be associated with important changes in the structures mediating these processes, such as the perirhinal cortex and the hippocampus.

Relationship between normal anatomical development and stages of memory processing

Studies investigating the normal anatomical development of the medial temporal lobe structures suggest that each matures at a different stage in development. The visual association areas, such as the perirhinal cortex, which support perceptual and encoding processes, are not fully developed at birth and their synaptic density does not reach adult-like morphology until nearly 24 months of age in humans (Huttenlocher, 1979; Huttenlocher, & Dabholkar, 1997) and around 4 months of age in monkeys (Webster et al., 1991; Rodman et al., 1991). By contrast, the hippocampus, which supports

consolidation, also shows some levels of protracted maturation (Seress & Abraham, 2008). While neurogenesis in the CA fields is mostly complete by birth, neurogenesis in the dentate gyrus, which receives most of the inputs, is not mature for several postnatal months. In addition, the direct connection between the entorhinal cortex and CA1 is mature by 1 month, but the connections within the trisynaptic circuit and to the prefrontal cortex take over a year to mature.

This pattern of maturation within the temporal lobe structures may support some of the important changes found in incidental recognition memory over age. Data from animals with neonatal hippocampal and perirhinal lesions provide some support to this proposal. First, the presence of robust novelty preference at 1.5 and 6 months of age after neonatal hippocampal lesions indicates that other medial temporal lobe structures can support the early developing recognition memory abilities in the absence of a functional hippocampus. This conclusion is substantiated by weaker novelty preference found in animals with neonatal damage to the perirhinal cortex as early as 1.5 months of age as compared to controls. Second, at 18 months, the deficit in recognition memory found at the longest delays after neonatal hippocampal lesions but not at the shorter delays is in line with the findings reported in animals with hippocampal lesions in adulthood and differs from the deficit found at all delays for animals with neonatal perirhinal lesions. Consequently, neonatal perirhinal and hippocampal lesions have differing effects on incidental recognition memory across development. The perirhinal cortex appears to be necessary for robust novelty preference at all ages and delays as short as 10 s, whereas the hippocampus does not seem to be involved until around 18 months of age.

Thus, by 18 months of age, the hippocampus begins to interact with the perirhinal cortex to play a role in incidental recognition memory. It will be important to see how the perirhinal group performs on the VPC task as adults in order to determine if their preference for novelty continues to weaken, or if it improves, suggesting that the hippocampus could take over some of the function after it fully matures.

Another interesting finding from the current studies is that animals with neonatal hippocampal lesions showed only transient recognition memory impairment with Color stimuli at 18 months that is not seen when the animals reached adulthood. What might account for the better performance at 48 months than at 18 months? It is possible that as the hippocampus matures, it begins to interact with medial temporal cortical areas to support recognition memory processes. However, anatomical changes are still occurring past 18 months, so that with further maturation, it may begin to disengage from these incidental recognition processes under certain conditions, such as when the stimuli are very easy to discriminate, but not when they share many overlapping features (Zeamer & Bachevalier, 2009b). This proposal is supported by the decreased preference for novelty found in animals with neonatal perirhinal lesions relative to animals with neonatal hippocampal lesions at both 6 and 18 months of age. One way to test this idea in the future would be to test animals with neonatal lesions of the hippocampus on an incidental object recognition memory task, such as VPC at 18 months of age using stimuli with many overlapping features to determine if they show weaker preference for novelty at delays shorter than 120 s. This would suggest that the hippocampus plays a bigger role in incidental recognition memory as it begins to functionally mature, but that it disengages itself as it begins to reach adult levels of maturity.

Taken together, these findings add further support to the idea that the early developing brain may use different neural pathways to support the same cognitive functions in adults (Goldman and Rosvold, 1972; Webster et al., 1995; Bates, 2004; Ciesielski, Lesnik, Savoy, Grant, & Ahlfors, 2006; Zeamer and Bachevalier, 2009b). In addition, after early neonatal insult, there may be a change in the underlying normal circuitry to support these same processes. For example, recent work in intrauterine growth restricted (IUGR) humans showed that ERP patterns in an auditory recognition task (familiar mother's voice versus novel stranger's voice) were distinctly different from those of normally developing infants (Black, deRegnier, Long, Georgieff, & Nelson, 2004), suggesting there was a change in the neural circuits supporting this type of memory after IUGR. Thus neonatal insult to the brain causes a change in circuitry that would normally develop to support adult cognitive abilities.

Developmental Amnesia

The current data could also help resolve some debate in the literature concerning the lack of recognition memory deficit following developmental amnesia in humans. Hypoxic ischemia in early childhood results in selective damage to the hippocampus associated with severe developmental amnesia. Patients with developmental amnesia have profound deficits in declarative memory processes, specifically recall, yet have fairly intact recognition memory (Vargha-Khadem, Gadian, & Mishkin, 2001; de Haan, Mishkin, Baldeweg, & Vargha-Khadem, 2006; Adlam, Malloy, Mishkin, & Vargha-Khadem, 2009, see Manns, Hopkins, Reed, Kitchener, & Squire, 2003 for impairment in both recollection and familiarity). This is supported by studies in patients like Jon, who

has developmental amnesia (Baddeley, Vargha-Khadem, & Mishkin, 2001; Brandt, Gardiner, Vargha-Khadem, Baddeley, & Mishkin, 2009). Jon shows relatively intact item recognition memory, but impaired recollection. In addition, an ERP study found that Jon shows the normal ERP signature associated with familiarity, but lacks the one associated with recollection (Dezel, Vargha-Khadem, Heinze, & Mishkin, 2001). Mishkin and colleagues (1997) proposed that recognition memory in patients with developmental amnesia could be supported by spared rhinal cortices, which are now thought to support familiarity processes (Brown & Aggleton, 2001). In addition, similar to the spared recognition memory abilities in patients with developmental amnesia, animals with neonatal hippocampal lesions also demonstrated spared object recognition abilities as measured by the delayed nonmatching-to-sample and the object memory span tasks (E. Heuer and J. Bachevalier, unpublished data). Thus, the current findings of spared recognition abilities in monkeys with neonatal hippocampal lesions further support the idea that neonatal damage to the hippocampus spares familiarity based item recognition memory under certain conditions. In addition, the impaired recognition memory abilities of animals with neonatal perirhinal lesions supports Mishkin and colleagues' (1997) argument that familiarity based recognition memory is supported by the perirhinal cortex across development even in the absence of a functional hippocampus.

Role of the hippocampus in stimulus feature ambiguity

Monkeys with neonatal lesions of the hippocampal formation also showed strong novelty preference during adulthood when tested using Color and BW stimuli, and performed similarly to animals with adult lesions of the hippocampus tested with the

same stimuli under the same conditions (Zeamer & Bachevalier, 2009b). However, when tested with more difficult to discriminate BW-OF stimuli, animals with neonatal hippocampal lesions performed at chance with the longest delay of 120 s, but showed novelty preference as strong as the control group after a 10 s and 60 s delay. These findings support previous evidence showing that the hippocampus is engaged in recognition memory when the stimuli share many overlapping features (Ranganath et al., 2000; Holdstock et al., 2002; Pihlajamaki et al., 2004; Danckert et al., 2007; Bakker et al., 2008; Zeamer and Bachevalier, 2009b). However, animals with neonatal lesions of the hippocampus performed better than animals with adult lesions of the hippocampus at the two shorter delays of 10s and 60s (Nemanic et al., 2004; Zeamer and Bachevalier, 2009b). This sparing of preference for novelty suggests that the remaining cortical areas may remain plastic through development to mediate some of the recognition memory processes normally mediated by the hippocampus in adulthood. Interestingly, it has recently been shown that patient Jon showed also an impairment in the VPC task at the long delays when tested with difficult stimuli sharing many overlapping features (M. Munoz and F. Vargha-Khadem, personal communication). This finding provides further support to the idea that the hippocampus may only be involved in recognition memory processes when the task demands become too difficult for a simple familiarity judgment, such as when the stimuli are difficult to discriminate.

Can other structures support recognition memory?

While animals with neonatal lesions of the perirhinal cortex showed weaker preference for novelty relative to controls at all ages, they still performed above chance,

unlike animals with adult lesions of the perirhinal cortex. What other structures could account for this sparing of performance?

One candidate is area TE, which is adjacent to the perirhinal cortex and is highly interconnected. Area TE is involved in visual object processing and is considered the final stage in visual processing (Ungerleider & Mishkin, 1982). Adult lesions of TE cause severe deficits in recognition memory, yet monkeys with neonatal lesions of TE show reorganization and remarkable sparing relative to animals with adult lesions (Webster, Ungerleider & Bachevalier, 1995; 1991; Webster, Bachevalier, & Ungerledier, 1995; Buffalo et al., 1999; 2000). Thus, in the absence of a functional perirhinal cortex early in development, area TE may be able to support these early developing recognition memory processes.

Another possibility is that animals with neonatal perirhinal cortex lesions may be able to maintain the information in working memory using prefrontal strategies to perform well on the task. Anatomical studies have found a direct connection between both the entorhinal cortex and the hippocampus, and the prefrontal cortex (Insausti et al., 1987; Barbas & Blatt, 1995), suggesting that these structures work together in an integrative fashion to retrieve stored memories. Several electrophysiological and imaging studies have shown that medial temporal structures play a role in visual working memory (see for review Ranganath, 2006) and that the prefrontal cortex works in a top-down manner to maintain the complex images that are initially processed within the perirhinal cortex and hippocampus. Ranganath's model argues that this would support the activation of visual object representations relevant to the task at hand while inhibiting representations of objects that would distract from the task at hand. Thus, it is possible

that in the absence of a fully functional hippocampus, early developing recognition memory processes are supported by the perirhinal cortex and area TE, but the circuits between the entorhinal cortex and the prefrontal cortex would be sufficient to support some level of recognition memory at a later age when the prefrontal cortex matures, though it would not be as efficient as the direct connections from the hippocampus, thus recognition memory abilities would not be as strong as in a normal subject.

The familiarity recollection debate

Over the last decade, there has been a debate as to the role of the hippocampus and surrounding cortical areas in two components of recognition memory: familiarity and recollection. Several researchers argue that the hippocampus supports recollection and the surrounding cortex supports familiarity (Aggleton and Brown, 2001; Eichenbaum, 2007), while others argue that the hippocampus can support both (Squire et al., 2007). Several recent imaging studies support such a distinction (for review see Diana, Yonelinas, & Ranganath, 2007). For example, a patient with damage to the perirhinal cortex but not the hippocampus was impaired on familiarity judgments in the remember/know paradigm, but showed intact recollection responses (Bowles, et al., 2007). However patients with selective hippocampal damage that spares the perirhinal cortex, like Patient Jon, show impaired recollection but relatively intact recognition (Baddeley et al., 2001; Holdstock et al., 2002; Mayes et al., 2004; Brandt et al., 2008). The current data further supports such a distinction because animals with neonatal perirhinal cortex lesions were impaired on a test of familiarity (VPC) whereas animals with neonatal lesions of the hippocampus showed relatively intact novelty preference on the same task. However, this pattern of results would need to be confirmed when animals with neonatal lesions of the perirhinal cortex reach adulthood.

Conclusions

In summary, the current data show that incidental recognition memory processes are available early in development, and are the first to demonstrate that these processes

are in part dependent on the integrity of the medial temporal lobe structures at such an early age. Whereas the perirhinal cortex is necessary to support these processes early in development, the hippocampus does not begin to interact with the medial temporal cortical areas until around 18 months of age to support the same processes. In addition, these findings add further support to the idea that the neural pathways supporting recognition memory processes early in development may be distinctly different from those that support the same cognitive processes in adults (Goldman and Rosvold, 1972; Webster et al., 1995; Bates, 2004; Zeamer and Bachevalier, 2009b) and may help to better understand findings from patients with developmental amnesia. Finally, the current data add further support to a familiarity/recollection dissociation between the perirhinal cortex and hippocampus. However, further testing during adulthood on animals with neonatal lesions of the perirhinal cortex on the VPC task with varying feature ambiguity is necessary to determine if sparing of incidental recognition memory function will remain, or if with further maturation, animals will grow into a more profound recognition memory impairment.

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