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Impaired cognitive processes associated with memory loss after neonatal perirhinal lesions in rhesus macaques

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Impaired cognitive processes associated with memory loss after neonatal perirhinal lesions in rhesus macaques

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An abstract of
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Abstract

Impaired cognitive processes associated with memory loss after neonatal perirhinal lesions in rhesus macaques

By Alison R Weiss

The perirhinal cortex (PRh) is an area in the medial temporal lobe that receives inputs from the ventral visual stream, and projects to the entorhinal cortex, hippocampus, and the lateral prefrontal cortices. There is already preliminary evidence to suggest that the PRh is important to support the normal development of recognition memory (Weiss & Bachevalier, 2016; Zeamer et al., 2015). However there remain significant questions regarding the degree to which perceptual difficulties, retarded familiarity processes, impaired working memory, and difficulty resolving proactive interference may have contributed to the impaired performance of the Neo-PRh monkeys on the recognition tasks reported earlier. This dissertation presents three manuscripts that describe an attempt to clarify these issues. Study 1 provides evidence that neonatal PRh lesions had a significant impact on the development of familiarity mechanisms, but spared visual perception. Study 2 provides evidence that the same Neo-PRh lesions did not alter working memory processes per se, but rather increased the tendency to make perseverative errors. Study 3 provides evidence that impaired cognitive flexibility was a likely source of the increased perseverative errors made by Neo-PRh monkeys when performing WM tasks with proactive interference. Taken together, data from these studies advance our understanding of the fundamental cognitive processes that were impacted by the Neo-PRh lesions by highlighting the critical role this area plays in the development of recognition memory and executive function.

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General Introduction

Studying the development of medial temporal lobe functions is of major clinical interest given the learning and memory deficits that are associated with several developmental neuropsychiatric disorders (e.g. schizophrenia, autism, ADHD, Fragile X, Down's and Williams syndromes). These disorders share common factors (developmental components, genetic predisposition, and medial temporal lobe pathology), with similarly impaired cognitive functions but have different time courses and severity. Thus, a critical step towards creating effective interventions and treatments will require better understanding of the neural basis of perception, learning, and memory, and of the outcomes of early insult at different nodes along this network across development. Although a large body of work has linked structural and functional changes of the hippocampus to these disorders, more recent studies have indicated that the perirhinal (PRh) cortex, and its interactions with the hippocampus, plays a critical role in perception and memory (Murray & Wise, 2012; Ranganath, 2006; Suzuki & Naya, 2014) and may likewise be associated with the cognitive deficits in these disorders. This chapter is intended to provide an overview of the anatomical organization of the MTL, focusing on the PRh in particular, and a brief review of the current theories on the role of the PRh in the following cognitive functions: visual perception, familiarity judgments, working memory, and proactive interference. We will then briefly summarize the studies that have begun to characterize the effects of early insult to the PRh on memory processes across the lifetime in monkeys, and identify some of the weaknesses with the interpretation of the current data that have led to the 3 developmental studies

described in this thesis.

Anatomical Organization of the PRh

The PRh, defined by Brodman as areas 35 and 36 (Brodman, 1909), occupies the lateral bank of the rhinal sulcus and the medial section of the inferior temporal gyrus (Figure 1). Multiple sensory areas send projections to the PRh (Suzuki & Amaral, 1994; Suzuki, 1996), but its densest afferents originate in ventral visual temporal areas TE/TEO (Lavenex, Suzuki, & Amaral, 2004). The heaviest efferents of the PRh project to the anterior and lateral portions of the entorhinal cortex, with lighter projections to the distal section of CA1 and the proximal section of the subiculum (Insausti, Amaral, & Cowan, 1987; Suzuki & Amaral, 1994; Suzuki, 1996). The PRh has also bi-directional monosynaptic projections with the lateral prefrontal fields (BA 45, 47/12, 46, 9) (Kondo, Saleem, & Price, 2005), and multi-synaptic pathways through the thalamus connect the PRh to the medial prefrontal areas (mPFC) (Brodmann's areas 24, 25, 32, and 14) (Aggleton, 2012; Lavenex, Suzuki, & Amaral, 2002; Saunders, Mishkin, & Aggleton, 2005). It is also important to contrast the connections between the PRh and PFC with those between the hippocampus and PFC. The dlPFC projects back to the posterior hippocampus (Goldman-Rakic, Selemon, & Schwartz, 1984; Morris, Pandya, & Petrides, 1999) providing a potential top-down mechanism regulating hippocampal-dependent WM processes, but few of these afferents are reciprocated (for review see Aggleton, 2012). In sum, the PRh is a cortical area in the medial temporal lobe that receives inputs from the ventral visual stream (area TE/TEO), and projects to the entorhinal cortex,

hippocampus, and the lateral prefrontal cortices. The anatomical connectivity of the PRh places it in a unique position to coordinate neural representations of non-spatial visual information generated in the ventral visual stream with brain structures known to be important for memory (hippocampus) and executive function (PFC). Current evidence on the role of the PRh in visual perception, recognition, working memory, and proactive interference is reviewed below.

Cognitive Functions of the Perirhinal Cortex

Perirhinal Cortex and Visual Perception

Neurocognitive processes underlying visual perception begin in peripheral sensory organs (eyes), where visual stimuli are transduced into neural signals. Basic information about color, movement and form is coded and assembled into progressively complex representations, first in primary visual cortex (areas V1-V4) and then in the higher-order visual cortical areas of the dorsal and ventral visual streams. Seminal work demonstrated that dorsal visual areas in inferior parietal cortex allowed integration of visuospatial features to represent locations, whereas the ventral visual areas in inferior temporal cortex allowed integration of perceptual features to represent objects (Baizer, Ungerleider, & Desimone, 1991; Desimone, Schein, Moran, & Ungerleider, 1985; Haxby et al., 1991; Mishkin & Ungerleider, 1982; Ungerleider & Haxby, 1994; Wilson & Wilkinson, 2015). As the final station of this ventral pathway, it is in the PRh that a complete representation of a perceived object is realized (Murray & Wise, 2012; Murray & Richmond, 2001; Suzuki & Amaral, 1994b; Suzuki, 1996a). In this way, the PRh may

generate complex representations of objects from a compilation of perceptual features represented at lower levels of the visual hierarchy.

Most of the knowledge on the role of the PRh in perception comes from a body of work that has used neuroimaging and electrophysiological recording techniques to monitor PRh activity during perceptual tasks, or tested visual discrimination abilities after PRh damage. Neuroimaging data in healthy adults indicated that the PRh was highly active during perceptual task requiring the integration of visual features into configurally-based representations. This pattern of activation corresponds to the activity in inferotemporal cortical areas that have been historically linked to visual processes (Devlin & Price, 2007). Additionally, when subjects performed difficult figure-ground discrimination problems, the activity recorded in area V2 mimicked the activity usually recorded in PRh (Peterson et al., 2012). Taken together, these findings suggest that the PRh may interact with other cortical areas important for feature-based visual processing in order to construct visual representations from configurations of familiar features. Thus, it was not surprising that PRh lesions resulted in visual discrimination impairment when stimulus complexity was high or perceptual overlap between stimuli was extensive (i.e. feature ambiguity), although they spared the ability to discriminate two highly dissimilar stimuli (Barense, Ngo, Hung, & Peterson, 2012; Bussey, Saksida, & Murray, 2002, 2003, 2005, 2006; Hales, Broadbent, Velu, Squire, & Clark, 2015; Murray & Richmond, 2001). The specificity of the visual deficits following PRh lesions give credibility to the neuroimaging data suggesting the importance of this area in mechanisms of complex visual perception (Murray & Wise, 2012).

Perirhinal cortex and Familiarity Judgments

The PRh may not only be critical for object perception but its strong anatomical link with the entorhinal cortex and hippocampus has led some scientists to believe that the PRh may also be critical for memory processes, such as recognition. Recognition of a previously encountered item can be accomplished by recollecting the specific episode in which it was previously encountered, or by assessing the degree of familiarity with an object or event. In this way, mechanisms of recollection and familiarity are both capable of supporting recognition memory. Lesion studies in rodents and monkeys (Malkova, Bachevalier, Webster, & Mishkin, 2000; Meunier, Bachevalier, Mishkin, & Murray, 1993; Meunier, Hadfield, Bachevalier, & Murray, 1996; Mumby & Pinel, 1994; Nemanic, Alvarado, & Bachevalier, 2004; Wan, Aggleton, & Brown, 1999), as well as work with human neuropsychiatric populations (Bowles et al., 2007; Schoemaker, Gauthier, & Pruessner, 2014; Yonelinas et al., 2002) have all demonstrated the importance of the MTL structures for recognition memory. A number of newer studies have begun to provide evidence for the presence of a functional dissociation in the contribution of the hippocampus and the PRh to recognition memory, with the PRh being critical for familiarity judgments and the hippocampus associated with recollection. For instance, patients with extensive medial temporal lobe damage encompassing both the hippocampus and perirhinal cortex showed deficits in recollection and familiarity judgments (Yonelinas et al., 2002). However, selective damage to the hippocampus, sparing the PRh, impaired recollection but spared familiarity (Aggleton et al., 2005;

Mayes et al., 2004). Furthermore, electrophysiological recordings have demonstrated that neuronal firing in the PRh precedes cell firing in the hippocampus suggesting that there is a rapid familiarity signal mediated by the PRh, which is then followed by a late-onset recollection signal mediated by the hippocampus (Staresina et al., 2012; Staresina et al., 2013). Additional neuroimaging studies in healthy adults have shown that hippocampal activity increases in response to retrieval of information but not in response to judgments of familiarity (Eldridge et al., 2000; Vilberg & Rugg, 2007). In sum, the PRh appears to be important to support familiarity processes involved in recognition memory.

Perirhinal cortex and working memory

The PRh is involved in object recognition, and is critical for the integration of multiple features, across multiple domains, into an abstract view-invariant representation of a stimuli; in this fashion, it is hypothesized that PRh may also support contents of WM. The strong projection of the PRh with the lateral PFC could enable the PRh to interact with the PFC in support of memory processes that have been linked to this area, such as working memory.

Working memory (WM) involves the maintenance of a limited set of cognitive representations of objects, places, ideas, goals, or rules. Furthermore, these cognitive representations are kept active in a manner flexible enough to cooperate with simultaneous/parallel WM process that monitor or manipulate the representations being kept 'in mind,' (Cannon et al., 2005; D'Esposito, Postle, Ballard, & Lease, 1999;

Glahn et al., 2002; Owen, Herrod, & Menon, 1999; Petrides, 1991a, 1991b, 1995). In the last 50 years, overwhelming evidence has accumulated from human functional imaging (Cannon et al., 2005; D'Esposito et al., 1999; Glahn et al., 2002; Owen et al., 1999) and electrophysiological and lesion studies in monkeys (Kowalska, Bachevalier, & Mishkin, 1991; Miller & Buschman, 2013; Miller & Cohen, 2003; Mishkin & Manning, 1978; Passingham, 1975; Petrides, 1991a, 1991b, 1995) to indicate the importance of the ventrolateral prefrontal cortex (vlPFC) for WM maintenance processes and the dorsolateral prefrontal cortex (dlPFC) for higher-order WM monitoring/manipulation processes. However, more recent studies suggest that the prefrontal cortex is part of a broader network of interconnected brain areas involved in WM (Constantinidis & Procyk, 2004). Specifically, MTL structures are also recruited during WM tasks (Davachi & Goldman-Rakic, 2001; Kimble & Pribram, 1963; Libby et al., 2012; Petrides, 2000; Ranganath et al., 2004; Stern et al., 2001). One MTL structure well positioned to play a role in WM processes is the PRh, mainly because of its direct reciprocal connections with lateral and orbital PFC fields (Lavenex et al., 2002; Saunders et al., 2005; Suzuki & Amaral, 1994).

Electrophysiological and functional imaging studies indicated increased activity in PRh during object-based WM tasks, and give credibility to the theory that this cortical area supports object representations used in WM. Specifically, cells in the PRh of adult macaques were highly activated during WM tasks requiring the temporary maintenance of object representations (i.e. small-set DNMS). Interestingly, this activity was not observed in another temporal visual area, area TE (Lehky & Tanaka, 2007). Likewise, 2-

Deoxyglucose imaging studies demonstrated increased PRh activity during a delayed object alternation task; a task requiring the maintenance and monitoring of information in WM. The same increase was not seen in the entorhinal cortex (Davachi & Goldman-Rakic, 2001). Taken together, these results point to a unique contribution of the PRh to performance on tasks that require the active/flexible representation of familiar objects, that is in fact strengthened by the concurrent lack-of-contribution of both the primary afferents (area TE/TEO) and primary efferents (ERh) to the PRh. However, no studies to date have directly addressed the contribution of the PRh to WM.

Perirhinal cortex and proactive interference

Proactive interference occurs when previously acquired information impedes the ability to learn or apply new information, and may result in behavior that is dominated by rules no longer appropriate to the current situation (Owen et al., 1993; Postle, et al., 2004; Ridderinkhof, et al., 2002). To resolve proactive interference the influence of formerly active, and now competing, response sets must be suppressed (Jha et al., 2004; Monchi et al., 2001). This requires the inhibition of behavioral responses based on "old" information, and a flexible shift of cognitive resources towards learning/remembering "new" information (Jha et al., 2004). These cognitive mechanisms are critical for performance on tasks requiring participants to flexibly update cognitive representations or shift response strategies, such as self-ordered pointing tasks and attentional set-shifting tasks (Collins et al., 1998; Petrides & Milner, 1982; Ross, et al., 2007). Lesion studies in monkeys have already demonstrated that behavioral inhibition is supported

by the OFC, whereas cognitive flexibility is supported by the ventrolateral and medial PFC (Bissonette et al., 2013; Burnham et al., 2010; Dias et al., 1997; Monchi et al., 2001; Rogers et al., 2000) Given that the PRh has robust interconnections with all these PFC areas (Barbas & Pandya, 1989; Lavenex et al., 2002; Petrides & Pandya, 2002; Suzuki & Amaral, 1994, 1994), its contribution to in mechanisms underlying cognitive flexibility and/or behavioral inhibition is expected. There is already evidence that rhinal cortex damage in adulthood impairs performance on reversal-learning tasks, suggesting that the PRh could be important for mechanisms of behavioral inhibition (Hampton & Murray, 2002; Murray et al., 1998). In contrast, preliminary evidence on the effects of extended MTL damage in adulthood indicates that the MTL is not important to support performance on attentional set shifting tasks (Owen et al., 1991). However, no studies to date have directly addressed the role of the PRh in mechanisms of cognitive flexibility in adulthood, or during development.

Development of the PRh

As reviewed above, lesion studies in adult monkeys have provided strong evidence that the PRh plays a critical role in high-level perception and familiarity judgments supporting recognition. Yet, there are no studies that have investigated the role of the PRh in the development of these cognitive functions. Our laboratory has initiated a program of research to gain critical information on the role of the PRh in the development of memory functions. Current data obtained with the developmental studies in monkeys will be reviewed below together with additional information on the

anatomical development of the PRh.

Anatomical Development

Stereological examination of the neonatal rhesus brain have revealed that the cytoarchitectonic and anatomical characteristics of the PRh of primates appear nearly adult-like at birth (Berger & Alvarez, 1994). As early as 3 days postnatal, the fundus and lateral bank of the rhinal sulcus can already be identified by the distinct patterns of immunoreactive neurons seen in adults. Specifically, the distribution of parvalbumin and neurotensin expressing neurons is characteristically decreased in this area, similar to what has been observed in adult monkey and human brains (Pitkänen & Amaral, 1993; Tunon et al., 1992), and the density of catecholaminergic and serotoninergic inputs resembled the moderate increases seen in the adult monkey (Bakst, Morrison, & Amaral, 1985). Taken together, this evidence indicates that most of the morphological and neurochemical development of the PRh occurs before birth. The early maturation of the morphology of the PRh suggests that it should be functioning early in life to support cognition.

Functional Development

To gain knowledge on the development of PRh functions as well as to assess the effects of early PRh insult on the development of cognitive functions, we prepared infant monkeys with neonatal PRh lesions performed when the animals were 1-2 weeks old. These animals, as well as their age-matched controls, were given tasks measuring

recognition memory as well as working memory. This section briefly summarizes the findings of these studies and identifies some of the weaknesses in the current interpretations of the data that led to this dissertation project.

Recognition memory:

New data from a longitudinal study using rhesus macaques with neonatal PRh lesions (Neo-PRh) suggest that the PRh is capable of supporting recognition memory starting shortly after birth. Using an incidental recognition task (Visual Paired Comparison, VPC) with delays varying from 10-120s, normally developing infant monkeys (Neo-C) were able to reliably recognize stimuli across short and long delays, with the appearance of an adult-like delay-dependent memory performance emerging by 18 months (Zeamer, Heuer, & Bachevalier, 2010). In contrast, monkeys that received bilateral neurotoxic PRh lesions (Neo-PRh) showed a mild recognition impairment as early as 1.5 months. This memory loss became more severe during adolescence (18 months) and remained present in adulthood (48months) (Zeamer et al., 2015). Thus, the debilitating effects of the Neo-PRh lesions on the ability to recognize objects emerge early and are long lasting, suggesting that this cortical area may be critical to support the normal development of recognition memory processes.

The long-lasting impact of the Neo-PRh lesions on object recognition memory was also evident when the animals were tested as adults in a problem solving recognition memory task, Delayed Non-Match to Sample (DNMS), using delays varying from 10-120s. In this task, the same Neo-PRh monkeys were able to recognize familiar

objects after short (10s) delays, but were impaired when tested with delays 30s and longer. Thus, the pattern of recognition impairment appeared to be similar in the two tasks, although some interesting differences were noted.

First, performance on the VPC task lacked the delay-dependent forgetting curve characteristic of performance of animals with adult-onset PRh lesions; that is performance decreased as the length of the delays increased (Heuer & Bachevalier, 2011). Thus, although this delay-dependent performance was observed in the control animals, the scores of the Neo-PRh animals were worse than those of controls but were comparable at all delays. This pattern of results suggested that the cause of the poor performance on the VPC task by Neo-PRh animals may not be a loss of memory per se but rather difficulty with other processes, such as motivation to look at stimuli or perceptual ability. A lack of motivation could be rejected because Neo-PRh animals took the same amount of time to familiarize with stimuli and had the same amount of looking time during the two retention tests as the control animals, indicating that they were spending the same amount of time investigating the stimuli. However, a worsening of perceptual abilities after the neonatal PRh lesions could have affected novelty preference scores, especially given the critical role of the PRh in perceptual processes in adulthood. To test this proposal, the first aim of Study 1 was to characterize the ability of Neo-PRh monkeys and their controls to discriminate between complex visual stimuli. We conjectured that impairment in this task in the Neo-PRh animals would suggest that poor perceptual ability could account for the recognition impairment in the VPC task.

Second, comparisons between the effects of early-onset PRh lesions and adult-

onset PRh lesions can shed light on the role of the PRh during development. Adult-onset PRh lesions resulted in severe recognition deficits when measured either with the VPC task or the DNMS task (Meunier et al., 1993; Nemanic et al., 2004). The early-onset PRh lesions yielded a severe recognition deficit in the DNMS task (Weiss & Bachevalier, 2016) that was similar to the deficit seen after the adult-onset PRh lesions (Meunier et al., 1993). By comparison, the magnitude of the recognition deficits in the VPC task was significantly less severe after the Neo-PRh lesions than after the adult-onset PRh lesions (Zeamer et al., 2015; Nemanic et al., 2004). Thus, the evidence of a moderate functional sparing when recognition was measured with the VPC task contrasts with the lack of functional sparing when recognition was measured with the DNMS. Several factors may have led to this pattern of results.

First, as mentioned above, given the plasticity of the brain across development, it is possible that other MTL cortical areas could have compensated for the absence of the PRh. In addition, because the animals were tested at several time points during development with the VPC task, albeit with novel stimuli each time, practice on the task together with neural compensation mechanisms could have led to improve performance as the animals were re-tested as adults. Second, an important procedural difference between the two recognition tasks could have affected performance of the Neo-PRh animals. Although the VPC task uses a long familiarization time of 30s, the familiarization time in the DNMS task is much shorter and varies from 5-7s, i.e. the time required to the animals to displace the object and retrieve the reward. Given that the critical role of the PRh in familiarity judgments (Bowles et al., 2007; Yonelinas et al.,

2002), it is possible that Neo-PRh animals may require longer time to familiarize with a stimulus, in the absence of a functional PRh. To test which of these factors could have led to the mild sparing of recognition memory after Neo-PRh lesions, we began by investigating whether Neo-PRh lesions affected familiarity judgments. Thus, the second aim of Study 1 assessed familiarity judgments in animals with Neo-PRh lesions and their controls using a task with repeated familiarization exposures, the Constant Negative Discrimination Task (Browning et al., 2013).

Working Memory

As reported above, despite the strong anatomical connections between the PRh and the lateral prefrontal cortex, we still do not know whether the PRh cooperates with the PFC in support of executive functions. Thus, Study 2 began to evaluate whether neonatal lesions of the PRh may impact WM processes known to be mediated by the PFC. WM was assessed in the same animals with Neo-PRh lesions and their controls as they reached adulthood. WM was measured using three object-based tasks: the Session-Unique Delayed Non-Match to Sample (SU-DNMS), which requires the maintenance of object representations, the Object Self-Ordered (Obj-SO) task and the Serial Order Memory task (SOMT), which require maintenance and monitoring of the contents of WM. Comparisons of the results from the three WM tasks indicated that animals with Neo-PRh lesions did not have deficit in WM, but were sensitive to proactive interference and as a result made a greater number of perseverative errors as compared to controls.

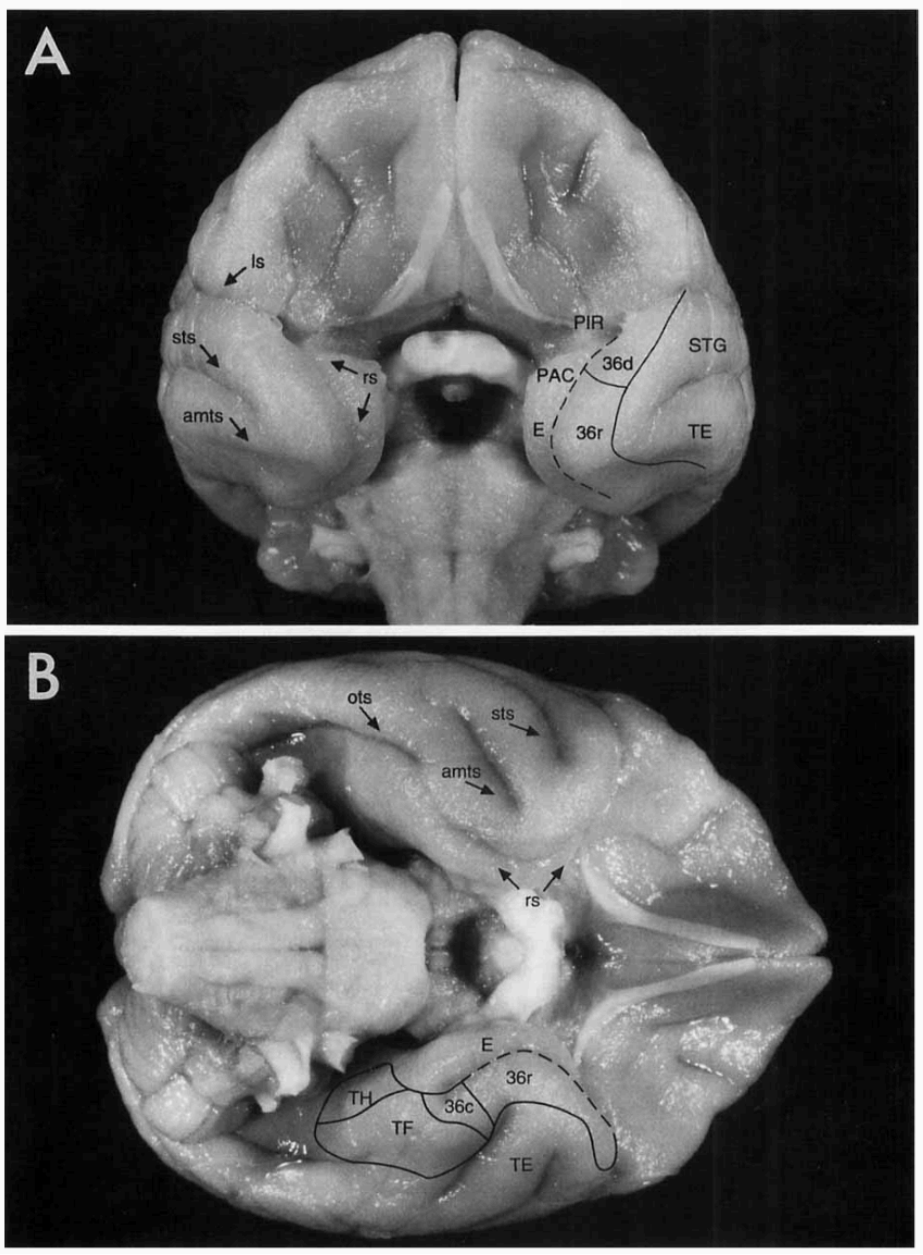
Finally, it became critical to investigate the source of the sharp increase in perseverative errors during WM tasks with high proactive interference. Such perseverations could relate to an inability to flexibly update cognitive representations or shift response strategies (i.e. cognitive flexibility or behavioral inhibition). Thus, Study 3 tested cognitive flexibility and behavioral inhibition, two important components that provide resilience against proactive interference, using the Intradimensional/Extradimensional (ID-ED) set-shifting task (Dias, Robbins, & Roberts, 1997).

Summary

There is already preliminary evidence to suggest that the PRh is important to support the normal development of recognition (Weiss & Bachevalier, 2016; Zeamer et al., 2015), and working memory (Weiss, unpublished masters thesis). However, as was described in the section above, significant questions remain to be explored regarding the degree to which the Neo-PRh lesions altered perceptual difficulties, familiarity processes, working memory, and inhibitory processes. To address these issues, I prepared three manuscripts describing the long-term impact of neonatal PRh lesions in adult rhesus macaques on visual perception and familiarity judgments (Study 1, paper in preparation), on working memory using tasks with or without proactive interference (Study 2, paper published), and on cognitive flexibility/behavioral inhibition (Study 3, paper in preparation). Taken together, data from these studies will advance our understanding of the sources of the impairments of Neo-PRh monkeys on recognition

(Weiss & Bachevalier, 2016; Zeamer et al., 2015) and working memory tasks (Weiss, unpublished masters thesis). Overall, this body of work represents a small, but important, step forward in our understanding of the cognitive processes supported by the PRh, and of the long-term impact of neonatal PRh insult in the development of these cognitive processes. The data may help to highlight the role of the PRh in the cognitive impairments seen in developmental neuropsychiatric disorders. Such knowledge may in the future lead to the development of more effective diagnoses, earlier interventions, and new therapies.

Figure 1: Perirhinal Cortex (areas 35/36) shown from on the rostral and ventral surface of the brain. Images were reproduced from Suzuki & Amaral, 1994a.



Manuscript in prep

Intact perceptual ability but impaired familiarity judgment after neonatal perirhinal lesions in rhesus macaques

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Abstract:

The perirhinal cortex has been thought to support high-level perceptual abilities as well as familiarity judgments (Suzuki & Naya, 2014; Tu, Hampton, & Murray, 2011; Warburton & Brown, 2010) that may affect recognition memory (Murray & Wise, 2012; Murray & Richmond, 2001). In this study, we tested whether poor perceptual abilities and/or a loss of familiarity judgment contributed to the recognition memory impairments reported in monkeys with PRh lesions created in infancy (Neo-PRh) (Weiss & Bachevalier, 2016; Zeamer et al., 2015). Perceptual abilities were assessed using a version of the Visual Paired Comparison task with highly similar black & white (B&W) stimuli (Experiment 1), and familiarity judgments were assed using the Constant Negative task requiring repeated familiarization exposures (Experiment 2). In Experiment 1, monkeys with Neo-PRh lesions were able to accurately recognize B&W stimuli after short delays, suggesting that these animals have perceptual abilities within the normal range. In Experiment 2, Neo-PRh monkeys were slower to acquire the Constant Negative task, suggesting these animals may require more exposures to an object before judging it as familiar. Taken together, these data help to account for the differential patterns of functional compensation on previously reported recognition tasks following neonatal versus adult-onset PRh lesions (Weiss & Bachevalier, 2016; Zeamer et al., 2015), and provide further support to the view that PRh is involved in familiarity processes.

Introduction:

The perirhinal cortex (PRh), a cortical area within the medial temporal lobe, provides representations of objects in support of visual perception and recognition memory (Murray & Wise, 2012; Ranganath, 2006; Suzuki & Naya, 2014). In adult monkeys, the impact of PRh damage on object recognition has been well characterized. Selective PRh lesions in adult monkeys resulted in delay-dependent impairment on the delayed nonmatching-to-sample task (DNMS) (Meunier et al., 1993), and abolished novelty preferences on the Visual Paired Comparison (VPC) (Nemanic, Alvarado, & Bachevalier, 2004). A growing number of studies have led researchers to propose that, in contrast to the hippocampus that is thought to support recollection, the PRh is thought to support recognition by detecting familiarity among objects (Eichenbaum et al., 2007; Schoemaker et al., 2014; Suzuki & Naya, 2014; Tu et al., 2011; Warburton & Brown, 2010; Yonelinas et al., 2002). Additional studies in monkeys with adult-onset PRh lesions revealed a mild perceptual impairment when the test stimuli were black and white (B&W) or had overlapping/similar features (Bussey, Saksida, & Murray, 2002, 2003, 2005, 2006; Hampton, 2005), suggesting that this cortical area may also contribute to higher-order visual processes. However, no studies to date have reported on the impact of *neonatal* PRh damage on similar visual processes.

New data from a recent longitudinal study tracking the development of memory in infant rhesus monkeys reported that bilateral neurotoxic PRh lesions created before 2 weeks of age (Neo-PRh) produced mild impairment in novelty preference on the VPC task that emerged at 1.5 months, became more severe during adolescence (18 months),

and remained present in adulthood (48 months) (Zeamer et al., 2015). Furthermore, this recognition memory impairment was also demonstrated when the same Neo-PRh monkeys were tested in another recognition memory task, the DNMS task, as adults. Animals with Neo-PRh lesions were able to normally learn the DNMS rule and accurately remembered familiar objects after short (10s) delays, but were increasingly impaired when tested with delays of 30s and longer (Weiss & Bachevalier, 2016; Zeamer et al., 2015). Taken together, these two studies provided further support to the view that PRh is involved in recognition and highlighted its early emerging role during development. However, as will be further discussed below, the difference in the magnitude of the deficit obtained with the two recognition tasks led us to question whether the deficit following the Neo-PRh lesions truly reflected truly a recognition deficit per se or could rather be interpreted as a deficit in familiarity judgment, or simply as poor perceptual abilities.

An interesting feature of the recognition memory impairment found after Neo-PRh lesions as measured by the VPC task was the lack of the typical delay-dependent forgetting curve reported after adult-onset PRh lesions (Nemanic et al., 2004). That is, the magnitude of the loss of novelty preference reduction after Neo-PRh lesions was comparable for all delays tested, from the shortest delay of 1s to the longest delay of 120s (Zeamer et al., 2015). One possible explanation for this pattern of results may relate to earlier findings demonstrating that the PRh contributes to perceptual abilities. Indeed, the PRh receives strong inputs from sensory cortical regions of the brain, with the densest afferents originating in ventral visual areas TE/TEO (Suzuki & Amaral, 1994;

Suzuki, 1996). In addition, lesion studies in adult monkeys show that selective damage to the PRh yields severe visual discrimination impairment, mainly when stimulus complexity is high or perceptual overlap between stimuli is extensive (i.e. feature ambiguity), but not when stimuli are distinctive (Bussey et al., 2002, 2003, 2005, 2006; Murray & Richmond, 2001). To test whether the poor perceptual abilities might be the source of the recognition memory deficits found after the Neo-PRh lesions, Experiment 1 assessed performance of the Neo-PRh monkeys and their controls on a new version of the VPC task using highly similar black and white (B&W) stimuli. We conjecture that the presence of a delay-dependent recognition deficit after Neo-PRh lesions in this new version of the VPC task (i.e. normal performance at short delays but impairment at long delays) will indicate that Neo-PRh animals have perceptual abilities in the normal range and that early PRh lesions impact recognition memory processes.

An additional important distinction in the recognition memory impairment following Neo-PRh lesions comes from a comparison of the effects of the Neo-PRh lesions on the two recognition memory tasks (i.e. VPC vs DNMS) with those obtained after adult-onset PRh lesions on the same two tasks. Adult-onset PRh lesions resulted in similar severe recognition deficit in the two tasks (Meunier et al., 1993; Nemanic et al., 2004). By contrast, the Neo-PRh lesions yielded different outcomes in the two tasks. Thus, although the magnitude of the recognition deficit in the DNMS task was similar after Neo-PRh or adult onset lesions (Weiss & Bachevalier, 2016), the magnitude of the recognition memory deficit in the VPC task was less severe after the Neo-PRh lesions than after the adult onset lesions (Zeamer et al., 2015). This difference in the magnitude

of impairment in the two recognition tasks may be due to the incidental nature of the VPC task that could have resulted in an apparent sparing of function as compared to the force-choice DNMS task. Alternatively, the different outcomes of the Neo-PRh lesions on the two recognition tasks may relate to important procedural differences in the familiarization phase. That is, in VPC, monkeys are familiarized with the stimulus for a cumulative looking time of 30s, whereas in DNMS the monkeys view the stimulus for the number of seconds it takes to displace an object (usually 3-7 seconds). Thus, greater familiarization with the sample stimuli in the VPC task could have resulted in stronger novelty preference than with DNMS. Length of familiarization phase has already been shown to impact the strength of novelty preference in the VPC task (Richmond, Sowerby, Colombo, & Hayne, 2004; Zeamer, Meunier, & Bachevalier, 2011), and in recent years, a number of electrophysiological studies in monkeys (Erickson, Jagadeesh, & Desimone, 2000; Liu & Richmond, 2000) and rats (Albasser et al., 2010; Zhu et al., 1997; Burke et al., 2012), as well as neuroimaging studies in humans (Dew & Cabeza, 2013; Guedj et al., 2010; Vilberg & Davachi, 2013) have linked activity of PRh neurons with mechanisms of familiarity judgment. To assess whether the different outcomes of the Neo-PRh lesions in the two recognition memory tasks relate to the time animals were given to familiarize with the sample stimulus, Experiment 2 measured performance of the animals with Neo-PRh lesions and their controls in a memory task, the Constant Negative task, requiring discrimination between novel objects and objects that the animals had experienced several times during the task (Browning, Baxter, & Gaffan, 2013). We conjecture that normal performance on this task will indicate normal

familiarity judgement after Neo-PRh lesions, whereas deficit will confirm that the difference in the magnitude impairment between the two recognition tasks may be due to an inability of Neo-PRh animals to form familiarity judgments.

General Methods:

All protocols were approved by the Institutional Animal Care and Use Committee at Emory University in Atlanta, Georgia and were in accordance with the NIH Guide for the care and use of Laboratory Animals (National Research Council (US), 2011).

Subjects

Sixteen adult rhesus macaques (*Macaca mulatta*), 8 female and 8 males, participated in this project. Fourteen received surgery on postnatal days 7-12, either bilateral ibotenic acid injections of the perirhinal cortex (Neo-PRh: 3 females, 3 males), or sham-surgery (Neo-C: 4 females, 4 males). Two additional monkeys (1 female, 1 male) did not undergo surgery but experienced the same rearing conditions (Neo-UC). One cohort of the Neo-C of the subjects (Neo-C-1 thru Neo-C-6) was born at the University of Texas M.D. Anderson Cancer Center Science Park (Bastrop, TX), and a second cohort of the Neo-C subjects (Neo-C-7 thru Neo-C-10) was born at the Yerkes National Primate Research Center (Lawrenceville, GA). At both institutions, monkeys received identical rearing procedures that included extensive opportunities for social interactions with age-matched peers and with human caregivers (for details see Goursaud & Bachevalier, 2007; Raper et al., 2013). Independent sample t-tests revealed no significant differences

between the GA and TX cohorts on any measure collected for this study. Additional independent-sample t-tests compared the Neo-C and Neo-UC groups and indicated also that these groups did not differ significantly. Thus, for all analyses reported here, data from all 10 of these control animals were combined into a single Neo-C group.

At the time of Experiment 1, the animals were an average of 6.9 years old. All monkeys had similar experience with cognitive testing, having previously completed tests of object recognition (Weiss & Bachevalier, 2016; Zeamer et al., 2015), working memory (Weiss, Nadji, & Bachevalier, 2016), and Emotional Reactivity (Alghrim, Raper, Johnson & Bachevalier, in prep). At the time of Experiment 2, the animals had reached 9.7 years old on average, and had additional experience with tasks of Concurrent Discrimination/Reinforcer devaluation (unpublished data), and Safety Signal Learning (unpublished data).

Neuroimaging and Surgical procedures

To create the selective PRh lesions, two series of MR images (structural T1 and Fluid Attenuated Inversion Recovery, FLAIR) were acquired pre-surgically to calculate injection sites, and served as a baseline for lesion extent measurements. The same series were repeated one week post-surgery to estimate extent of lesions. Images were acquired using a Siemens 3.0 T/90 cm whole body scanner and a 3" circular surface coil. First, a T1-weighted scan (spin-echo sequence, echo time [TE]=11 ms, repetition time [TR]= 450 ms, contiguous 1 mm section, 12 cm field of view [FOV], 256×256 matrix) was acquired in the coronal plane. Additionally, three fluid attenuated inversion recovery

(FLAIR) scans (3D T2-weighted fast spoiled gradient [FSPGR]-echo sequence, TE=2.6 ms, TR=10.2 ms, 25° flip angle, 12 cm FOV, 256×256 matrix) were obtained in the coronal plane at 3.0 mm (each offset of 1 mm posteriorly) throughout the brain.

Throughout the duration of the scans and surgery that followed, the animals were under gas anesthesia (1.0 –3.0%, v/v, to effect) and their head was secured in a stereotaxic apparatus. An IV drip containing dextrose and 0.45% sodium chloride was used to maintain normal hydration, a heating pad was placed under the animals to prevent hypothermia, and vital signs (heart and respiration rates, expired CO2, and temperature) were monitored until the monkey fully recovered from anesthesia.

Upon completion of pre-surgical scans, the animals were moved to the surgical suite where they were prepared for injections using aseptic surgical procedures. Three sites were selected bilaterally and their MR coordinates transformed into stereotaxic coordinates. These sites were spaced in 2mm intervals along the anterior-posterior axis of the PRh, and each received an injection of 0.4µl ibotenic acid (Biosearch Technologies, Novato, CA, 10mg/ml in PBS, pH 7.4) at a rate of 0.4µl/min. Sham-operated controls underwent the same anesthetic, imaging, and surgical procedures, except no needles were lowered in the brain. Recovery of the animals was closely monitored, and analgesic (acetaminophen, 10mg/kg, p.o.) was given QID for 3 days after surgery. Additionally, all of the animals received dexamethazone sodium phosphate (0.4mg/kg, i.m.) to reduce edema, and Cephazolin (25 mg/kg, i.m.) to prevent infection SID starting 12h prior to surgery and ending 7 days after.

Lesion Assessment

All monkeys are participating in an ongoing longitudinal study, and so post-mortem histological evaluations of the lesions are unavailable at this time. Instead, lesion extents were estimated using coronal FLAIR images acquired 1-week post-surgery. Ibotenic acid injection causes cell death and induces edema that is detected as hypersignals (increased white areas) on the FLAIR images. Using Adobe Photoshop, these areas of hypersignals were drawn onto corresponding coronal sections of a normal 1-week old rhesus monkey brain (J. Bachevalier, unpublished atlas). These images were imported into Image J® and the lesion surface area was calculated in pixel^2 for each slice. The volume of the lesion was then calculated by summing the surface area of the lesion on each coronal section and multiplying by image thickness (1mm). Finally, the percent damage to the intended area (PRh), and unintended damage to adjacent structures (visual areas TE/TEO, entorhinal and parahippocampal cortex, amygdala, and hippocampus), was calculated by dividing the volume of the lesion by the volume of each structure in the control atlas and multiplying by 100 (for details see Nemanic et al., 2004).

A summary of the extent of intended and unintended damage resulting from the ibotenic acid injections for each surgical case is presented in Table 1. Briefly, extensive bilateral lateral damage to the PRh was observed for all cases (average=73.60%, min=67.06%, max=83.34%). In addition, ibotenic acid injections caused mild unintended damage to the entorhinal cortex (average=20.57%, min=5.42%, max=34.49%). Figure 1 shows pre-surgical and post-surgical MR images of a representative case. Images from

additional cases have been previously published (Weiss & Bachevalier, 2016; Weiss et al., 2016; Zeamer et al., 2015; Alhgrim, Raper, Johnson & Bachevalier, in prep; Weiss, White & Bachevalier, in prep).

Experiment 1: Impact of stimulus similarity on incidental object recognition

As adults, monkeys with Neo-PRh lesions showed impaired novelty preference when tested with the VPC task (see Figure 3A reproduced from Zeamer et al., 2015). However, inspection of the pattern performance revealed that Neo-PRh monkeys performed similarly at all delays, indicating the absence of the typical forgetting curve (normal performance at short delays but impairment at longer delays) normally observed after adult-onset PRh lesions (Zeamer et al., 2015). This lack of delay-dependent performance suggests that the reduced novelty preference after Neo-PRh lesions may have resulted from impairment in processes other than memory. Given the evidence that PRh lesions alters perceptual processes in adults, Experiment 1 tested Neo-PRh animals and their controls on a version of the VPC task that used B&W stimuli designed to have overlapping features. We conjectured that normal performance at short delays after Neo-PRh lesions would indicate a lack of perceptual deficits.

Methods

Apparatus

During all testing sessions monkeys were seated in a custom-made Plexiglas primate chair. The subjects viewed stimuli on a 19” monitor placed approximately 40cm

away. To encourage the animals not to look away from the monitor, their head movements were restricted using a custom molded thermoplastic helmet (Machado & Nelson, 2011). An experimenter controlled the stimulus presentation via a Dell laptop connected to the monitor. A digital video camera was mounted above the monitor and focused on the eyes such that the experimenter had a clear view of the looking behavior throughout the entire testing session. Looking behavior during each testing session was recorded onto a memory card for later coding.

Task

The VPC task is a preferential looking paradigm that takes advantage of the natural inclination of monkeys to look at novel stimuli. In this version, the stimuli consisted of pairs of images of highly similar black and white objects and were identical to those used by Zeamer & Bachevalier (2013). Figure 2 provides examples of the stimuli, and a schematic representation of a VPC trial. Each trial consisted of a Familiarization phase and two Retention Tests. During the Familiarization phase, monkeys fixated on a centrally presented stimulus until a cumulative looking time of 30s was achieved. Next was the occurrence of a variable delay (10s, 60s, or 120s), followed by two 5-sec Retention Tests (each separated by a 5s delay) during which the familiarized object was paired with a novel of the same category, shape and color. Variable delays were randomly intermixed within a daily session. The left-right position of the novel and familiar stimulus varied pseudo-randomly across trials and was reversed between the first and second Retention Tests. Trials were separated by 30-

second inter-trial intervals during which the screen remained black and the monkey was offered a preferred treat (i.e. raisin, jelly bean, marshmallow). A white noise generator was used throughout testing to reduce external noise and minimize disruptions. Monkeys completed between 3-7 trials per testing day, and were tested until they completed 10 trials at each delay (30 trials total).

Data Analysis

Preferential looking towards the novel stimuli is an index for recognition of the familiarized stimulus. Novelty preference scores were calculated for each trial using frame-by-frame analysis of the eye movements recorded during testing (see details in Pascalis & Bachevalier, 1999). A trained observer (with inter-observer reliability: Pearson r = 0.931), who was blind to experimental condition and the location of the novel image, scored the videos. From each trial three measures were calculated: 1) *familiarization time*, defined as the time to accumulate 30s of fixation in the familiarization phase; 2) *total looking time*, defined as the total amount of time spent looking at each stimulus during both retention tests; and 3) *percent novel*, defined as the time spent looking at the novel stimulus during the two retention tests divided by the total looking time and then multiplied by 100. Trials for which the total looking time was less than 1s were excluded from the analysis, but this occurred on less than 6% of trials (30 out of the total 580).

Group X Delay ANOVAs, using repeated measures for the second factor, tested the effects of the lesion and delay-length on familiarization time, total looking time, and

novelty preference for the B&W stimuli. Planned independent-sample t-tests were subsequently used to compare scores between the Neo-C and Neo-PRh groups at each delay. To determine whether there were any female/male differences among the groups, all analyses were also run using sex as a second independent factor. None of the analyses revealed significant sex effects, and so both sexes were combined for all analyses reported in the Results section. For all ANOVAs, effect sizes were reported using partial eta squared (η_p^2). For all t-tests, effect sizes were reported using Cohen's *d* (d_{Cohen}).

To determine if the size of the lesion could have impacted performance on the B&W VPC, additional bivariate Pearson correlations were performed between extent of PRh damage, or unintended damage in the adjacent entorhinal cortex (ERh), and novelty preference at each delay.

Results

Familiarization Time

Analysis of the Familiarization Time revealed that Neo-C and Neo-PRh groups required similar amounts of time to accumulate the 30 seconds of looking time required during the familiarization phase of the B&W VPC task [Group: $F(1,14)=0.094$, $p=0.766$, $\eta_p^2=0.007$; Delay: $F(2,28)=1.382$, $p=0.268$, $\eta_p^2=0.090$; Group X Delay: $F(3=2,28)=0.376$, $p=0.690$, $\eta_p^2=0.026$].

Total Looking Time

Analysis of the Total Looking Time (TLT) measure indicated that the Neo-C group had significantly higher TLTs than the Neo-PRh group [$F(1,14)=8.264$, $p=0.012$, $\eta_p^2=0.371$]. Analyses also revealed a significant effect of Delay [$F(2,28)=12.637$, $p<0.001$, $\eta_p^2=0.474$], and a significant interaction [$F(2,28)=3.605$, $p=0.040$, $\eta_p^2=0.205$]. Planned independent-sample t-test revealed that group Neo-C had significantly longer TLTs than group Neo-PRh during all delays [10s: $t(14)=3.115$, $p=0.008$, $d_{Cohen}=1.609$; 60s: $t(14)=2.708$, $p=0.017$, $d_{Cohen}=1.398$; 120s: $t(14)=2.686$, $p=0.018$, $d_{Cohen}=1.387$]. Additional planned paired-sample t-tests indicated that the TLT of group Neo-C was significantly higher for the 10s delay than the 60s [$t(9)=4.386$, $p=0.002$, $d_{Cohen}=0.286$] and 120s [$t(9)=8.200$, $p<0.001$, $d_{Cohen}=0.441$], but did not differ between the 60s and 120s delays [$t(9)=1.495$, $p=0.169$, $d_{Cohen}=0.141$]. In contrast, TLT for the Neo-PRh group did not differ between any of the delay conditions [10s vs 60s: $t(5)=1.150$, $p=0.302$, $d_{Cohen}=0.359$; 10s vs 120s: $t(5)=1.513$, $p=0.191$, $d_{Cohen}=0.467$; 60s vs 120s: $t(5)=0.290$, $p=0.784$, $d_{Cohen}=0.106$].

Novelty Preference

The average novelty preferences of Neo-PRh and Neo-C groups are illustrated for each of the 3 delays in Figure 3B. A 2-way repeated measures ANOVA revealed significant main effects of Group [$F(1,14)=6.637$, $p=0.022$, $\eta_p^2=0.322$] on Novelty Preference, but no significant main effect of Delay [$F(2,28)=2.374$, $p=0.112$, $\eta_p^2=0.145$] and no interaction between these factors [$F(2,28)=0.908$, $p=0.415$, $\eta_p^2=0.061$]. Planned group comparisons of novelty preferences at each delay separately revealed significant

group differences in Novelty Preference at the 60s and 120s delays [60s: t(14)=2.582, p=0.022, d_{Cohen}=1.334; 120s: t(14)=2.358, p=0.033, d_{Cohen}=1.218], but not at the 10s delays [t(14)=0.989, p=0.339, d_{Cohen}=0.511].

Correlation with lesion extent

The extent of PRh damage was not correlated with novelty preference at any of the delays tested [1s: r=0.542, p=0.267; 10s: r=0.309, p= 0.551; 60s: r=-0.629, p=0.181; 120s: r=-0.415, p=0.414]. Similarly, the extent of unintended entorhinal cortex damage was not correlated with any measures of task performance [1s: r=0.372, p=0.527; 10s: r=0.328, p= 0.526; 60s: r=-0.122, p=0.817; 120s: r=0.667 p=0.148]. However, it must be acknowledged that the lesions in the Neo-PRh monkeys were similar in extent, ranging only between 70%-85% (see Table 1). This lack of variability most likely contributed to the lack of correlations between extent of lesions and task performance.

Summary:

Experiment 1 tested whether poor perceptual abilities contributed to the recognition memory deficits reported previously in the Neo-PRh monkeys (Weiss & Bachevalier, 2016; Zeamer et al., 2015) with a new version of the VPC task using perceptually similar B&W stimuli. The results indicated that the groups performed similarly when the delays were kept short (10s), and that Neo-PRh animals had significantly lower novelty preferences than controls when the delays were extended to 60s and 120s. Given the normal levels of novelty preference after the 10s delay, these

data suggest that the Neo-PRh animals have perceptual abilities within the normal range.

Experiment 2: Familiarity Discrimination

Adult monkeys with Neo-PRh lesions were impaired on two object recognition tasks: VPC and DNMS (Weiss & Bachevalier, 2016; Zeamer et al., 2015). However, compared with adult-onset PRh lesions, the Neo-PRh lesions resulted in a partial sparing of recognition memory when measured using VPC, but not when measured using DNMS. An important procedural difference between the two memory tasks is the length of the familiarization time. Although the VPC task uses 30s cumulative time, the DNMS task uses shorter time, that is the time the monkeys takes to displace the object (usually 3-7s). Therefore, one possible explanation for the recognition memory sparing observed with the VPC task is that Neo-PRh animals were exposed for longer amount of time to the stimulus. To test whether animals with PRh may require longer time to become familiarized with a stimulus, we used an object discrimination task, the Constant Negative task (Browning et al., 2013), which required them to discriminate a novel object from an object for which the animals have been familiarized with.

Methods

Subjects

All 6 of the Neo-PRh animals (Neo-PRh-1 - Neo-PRh-6) participated in this experiment. At the time of this experiment, only 3 of the Neo-C animals were available

to participate (Neo-C-1, Neo-C-7, and Neo-C-9).

Apparatus and Stimuli

In our version of the Constant Negative task, monkeys were positioned in the Wisconsin General Testing Apparatus (WGTA) facing a tray with 3 recessed food wells (2cm diameter, 1cm deep, spaced 13cm apart). A collection of 960 junk objects that differed in size, shape, color, and texture were used as stimuli and sampled without replacement until completion of the task. Correct responses were rewarded with preferred food rewards (i.e. mini-marshmallow, jelly bean, M&M etc.). Animals were mildly food deprived prior to testing, and their weight monitored carefully and maintained at least 85% of normal body weight.

Task

The Constant Negative task was based on the paradigm developed by Browning, Baxter & Gaffan (2013), and is illustrated in Figure 4. During a daily session, monkeys were given a set of 60 unique discrimination problems in which they chose between two objects. For each problem, one object was designated the unrewarded "constant negative" stimulus (S-) and another never-before-seen (novel) object was designated the rewarded stimulus (S+). The 60 S- objects were presented once during every session, and became familiar over several days of testing. In contrast, the S+ objects presented together with the S- were always novel, and were drawn from the pool of the remaining 900 junk objects without replacement. The order in which the 60 S- stimuli were

presented was shuffled each session and a 30-s intertrial interval was used. Monkeys were trained daily in this task until they reached the learning criterion of 90% (54/60) correct followed by a score of 85% (51/60) or better the subsequent training session.

Comparison with Concurrent Discrimination task

Like the VPC and DNMS tasks, the Constant Negative task is designed to encourage the discrimination of novel objects among a set of familiar objects. However, the use of alternative strategies may allow good performance on the task. For example, although it is possible is that performance was driven by a mnemonic-based strategy of responding to novel stimuli (accomplished by using memory traces to discriminate novel from familiar stimuli), performance could also have been driven by "habit" learning systems (see Bachevalier, 1990). If so, monkeys may have instead learned to avoid the Constant Negative objects because they were consistently associated with no reward, as is the case in traditional habit learning paradigms, such as the Concurrent Discrimination task (as in Kazama, Davis, & Bachevalier, 2014), rather than because the familiar objects were explicitly remembered and avoided. Given that the Neo-PRh and Neo-C groups had also previously completed a 60-pair Concurrent Discrimination task using the same testing apparatus and similar stimulus materials as the Constant Negative task, we compared the scores the animals obtained in both tasks to gain insight into the types of strategies the animals may have used to support their performances.

Data analyses

The numbers of trials and errors to reach the learning criterion were used as the dependent measures, and independent sample t-tests were used to compare the performance of the Neo-PRh monkeys with that of the Neo-C. The same analyses were also re-run using sex as a second independent factor to determine whether there were any female/male differences among the groups. None of the analyses revealed significant sex effects, and so both sexes were combined for all analyses reported in the Results section.

Additionally, we calculated the learning curves for each group to investigate whether the speed at which the Neo-PRh animals became familiar with the S- objects differed from that of the Neo-C animals. A multiple regression model was used to determine whether the slopes of the learning curves differed between the two groups.

Bivariate Pearson correlations were performed to examine the relationship between the scores on the Constant Negative task and the extent of PRh damage or unintended damage in adjacent areas.

Finally, to compare performance on the Constant Negative task with that of the Concurrent Discrimination task, a Group x Task repeated measures ANOVA was used to compare the number of errors needed to reach the same learning criterion (54 out of 60) on the two tasks.

For all ANOVAs, effect sizes were reported using partial eta squared (η_p^2). For all T-tests, effect sizes were reported using Cohen's *d* (d_{Cohen}).

Results:

The average number of errors made by the Neo-C and Neo-PRh groups (92 and 110 respectively) before reaching the learning criterion did not significantly differ [$t(7)=-1.07$, $p=0.321$, $d_{Cohen}=0.755$; see Figure 5B]. However, as illustrated in Figure 5A, the Neo-PRh group required significantly more trials than the Neo-C group (450 and 320 respectively) to achieve the learning criterion [$t(7)=-2.54$, $p=0.039$, $d_{Cohen}=1.798$].

A multiple regression model using Group, Session, and their interaction, was found to significantly predict Errors [$F(3,67)=39.726$, $p<0.0001$, $R^2=0.640$]. Results of this analysis indicated that Group [$\beta=-0.578$, $t(67)=-3.584$, $p=0.001$] and Session [$\beta=-1.405$, $t(67)=-7.102$, $p<0.001$] were both reliable predictors of Errors on the Constant Negative task. Importantly, the interaction between Group and Session was also significant [$\beta=0.967$, $t(67)=3.637$, $p=0.001$], suggesting that the slopes of the learning curves (Figure 6) differed between the groups, with the Neo-C group having steeper learning curves than the Neo-PRh group.

Correlations with lesion extent:

The extent of PRh damage was not correlated with any measures of task performance [Errors: $r= -0.557$, $p= 0.251$; Trials: $r= -0.574$, $p= 0.234$]. Similarly, the extent of entorhinal damage was not correlated with any measures of task performance [Errors: $r= -0.186$, $p= 0.724$; Trials: $r= -0.716$, $p= 0.109$]. This indicates that the extent of the damage caused by the neonatal ibotenic acid injections is not likely to be related to task performance.

Comparisons with Concurrent Discrimination task:

A comparison of the number of errors each group made in the Constant Negative and Concurrent Discrimination tasks is illustrated in Figure 7. The Group x Task interaction [$F(1,7)=8.346$, $p=0.023$, $\eta_p^2=0.544$], as well as the main effect of Task [$F(1,7)=10.418$; $p=0.014$, $\eta_p^2=0.598$] reached significance, but the effect of Group did not [$F(1,7)=5.050$, $p=0.059$, $\eta_p^2=0.419$]. Planned paired-sample t-tests revealed that the Neo-PRh group made similar numbers of errors in both tasks (average = 110 vs 117 errors for Constant Negative and Concurrent discrimination tasks respectively; $t(5)= -0.364$, $p=0.731$, $d_{Cohen}=0.217$). By contrast, the Neo-C group made fewer errors in the Constant Negative task than in the Concurrent discrimination task (average = 92 vs 212, respectively), but this difference did not reach significance, [$t(2)=-2.739$, $p=.111$, $d_{Cohen}=2.452$]. Finally, planned comparisons indicated that although both groups had the same number of errors in the Constant Negative task, group Neo-PRh made significantly less errors than group Neo-C on the Concurrent Discrimination task [$t(7)=2.919$, $p=0.022$, $d_{Cohen}=2.064$].

Summary:

Experiment 2 assessed the effects of Neo-PRh lesions on familiarity judgment using the Constant Negative task. Neo-PRh monkeys required significantly more trials to reach the learning criterion, yet they made similar numbers of errors as controls. Further analysis revealed that the rate at which the Neo-PRh animals became familiar with the Constant Negative S- objects was slower than the Neo-C animals. These

differential learning rates suggest that Neo-PRh monkeys were slower to familiarize with the constant negative (S-) objects as compared to controls. Finally, unlike control animals, those with Neo-PRh lesions performed similarly in both the Constant Negative and Concurrent Discrimination tasks.

Discussion

The study revealed several original findings on the effects of neonatal PRh lesions. First, although these early lesions had minimal, or no, impact on perceptual abilities, they did affect the speed with which animals became familiar with stimuli. Second, a comparison between performance on the Constant Negative task and the Concurrent Discrimination task also suggests that animals with Neo-PRh lesions may have developed strong habit learning strategies to compensate for their poor recognition memory abilities. These findings are discussed in turn.

Perirhinal cortex and perceptual abilities

Previous lesion studies in adult monkeys have provided evidence for a critical role of the PRh in perceptual abilities (for review see Murray, Bussey, & Saksida, 2007). Experiment 1 sought to determine whether neonatal lesions of the PRh will lead to similar perceptual impairment. Using a version of the VPC task with highly similar B&W stimuli, we found that adult monkeys with neonatal PRh lesions displayed normal levels of novelty preference on the B&W VPC task after short delays, but were impaired as compared to controls when delays extended to 60s and 120s. This pattern of

performance disproves the proposal that perceptual impairments may account for the poorer recognition memory performance of Neo-PRh monkeys reported earlier (Weiss & Bachevalier, 2016; Zeamer et al., 2015). Instead, these data indicate that Neo-PRh monkeys have perceptual abilities within the normal range but have impaired recognition memory.

The normal perceptual ability after neonatal PRh lesions contrasts with data from a series of studies in adult-onset PRh lesions reporting perceptual impairments using B&W photographic stimuli with highly overlapping features (Bussey et al., 2002, 2003, 2005, 2006; Hampton, 2005). One possible explanation for the lack of perceptual impairments after Neo-PRh lesions may relate to the stimuli used, which may not have been sufficiently ambiguous as compared to those used in adult-onset lesions in both monkeys and humans. For example perceptual impairment in adult human neuropsychiatric patients with PRh damage has been reported when tested using abstract B&W stimuli (Barense et al., 2012; Neusome, Duarte & Barense, 2012), but not color (Hales et al., 2015). However, perceptual impairment was found in patients with brain damage that included the PRh when tested with B&W line drawings similar to those used in the current study (Newsome et al., 2012). Thus, differences in the types of stimuli used may not entirely explain the different outcomes between early-onset and adult-onset PRh lesions in perceptual abilities. Another, more likely, interpretation relates to the early timing of the PRh lesions in the current study. Given the levels of neural plasticity normally occurring during infancy (for reviews see Kolb & Gibb, 2007; Takesian & Hensch, 2013), it is possible that other structures could have compensated

for the perceptual abilities in the absence of a fully functional PRh. Neuroimaging data in healthy adults have indicated that V2 activation mimics the activity of the PRh during perceptual tasks that involve difficult visual discriminations (Peterson et al., 2012). These data highlight the broader network of brain areas that are recruited during perceptual learning tasks, and point to another structure that could potentially mediate visual processing after neonatal PRh lesions.

Perirhinal cortex and familiarity judgments

Comparisons between the effects of adult-onset and early-onset PRh lesions on the VPC and DNMS tasks reported earlier indicated that the magnitude of recognition deficit in the DNMS task was similar after the early-onset and adult onset lesions (Meunier et al., 1993; Weiss & Bachevalier, 2016). However, the magnitude of the recognition memory deficit in the color VPC task was less severe after the Neo-PRh lesions than after the adult-onset lesions (Zeamer et al., 2015). Thus, greater sparing of recognition memory was apparent when the Neo-PRh monkeys were tested in the VPC than on the DNMS. Given that the familiarization phase in DNMS was much shorter than in VPC, Experiment 2 explored the proposal that the Neo-PRh lesions resulted in poor familiarization abilities that resulted in more severe impairment in the DNMS than in the VPC. Using the Constant Negative familiarity discrimination task, the data demonstrated that the Neo-PRh monkeys required more exposures to objects before judging them as familiar and help to account for the functional compensation observed on the VPC task but not on the DNMS task (Weiss & Bachevalier, 2016; Zeamer et al.,

2015).

Furthermore, the mild effects of the Neo-PRh lesions on familiarity in the Constant Negative task could have resulted from the use of cognitive strategies other than recognition that may have partially masked more severe familiarity impairments. For example, developing a habit of avoiding the familiar objects, not because they are recognized but because they are consistently associated with non-reward, could also support performance on the Constant Negative task. To examine this possibility, performance of Neo-PRh and Neo-C animals on the Constant Negative task was compared to performance of the same animals on the 60-pair Concurrent Discrimination task, a habit-learning paradigm (Mishkin et al., 1984). Neo-C monkeys made fewer errors on the Constant Negative task than on the Concurrent Discrimination task, suggesting that they were using different strategies to solve the two tasks. This finding corroborates prior research reporting that healthy adult monkeys also make fewer errors on the Constant Negative task than on the Concurrent Discrimination task (Browning et al., 2013), and indicates that normal monkeys tend to use familiarity-based strategies to solve the Constant Negative task but habit-based strategies to solve the Concurrent Discrimination. In contrast, the Neo-PRh group made a similar numbers of errors on the Constant Negative and Concurrent Discrimination tasks, suggesting that these monkeys may have used habits to guide their responses on both tasks. An important additional finding is that the Neo-PRh monkeys make significantly fewer errors on the 60-pair Concurrent Discrimination task than Neo-C monkeys, suggesting that the early lesion facilitated the acquisition of habits. Therefore, another

interpretation of the mild impairment of the Neo-PRh monkeys on the Constant Negative task is that Neo-PRh monkeys may have developed more robust habit-learning strategies than control animals and these strategies may have helped them to compensate for their poor recognition memory to solve the Constant Negative Task. Additional studies are needed to fully assess the source of the impairment in the Constant Negative task and to disentangle the different competing cognitive systems available for performance on this memory task.

Conclusion:

Data from the current study enhanced previous interpretations of the pattern of recognition impairment reported in adult rhesus monkeys with neonatal PRh lesions (Weiss & Bachevalier, 2016; Zeamer et al., 2015). Taken together, the body of work from the neonatal perirhinal lesions provides further support to the view that PRh contributes to recognition via mechanisms of familiarity.

Figure Captions:

Figure 1. Pre-surgical structural T1-weighted images (left column) and Post-surgical FLAIR images (right column) at three rostro-caudal levels through the perirhinal cortex from a representative case (Neo-PRh-5). Post-surgical FLAIR images show regions of hypersignal (white areas) indicative of edema and cell damage caused by the injection of ibotenic acid. In the left column, arrows point to the rhinal sulcus. In the right column, arrows point to regions of hypersignals.

Figure 2. In [A], a representative trial of the VPC task that consisted of a cumulative familiarization phase of 30s followed by delays from 10, 60 and 120s. After a short 5s interval, two Retention tests of 5s each were given separated by a 5s delay. In the Retention tests, the now familiar stimulus was paired with a novel, but similar, stimulus. 30s inter-trial-intervals separated the trials. Examples of the stimuli used in this task are shown in [B].

Figure 3. Average novelty preference (±SEM) across the delays in animals with neonatal perirhinal cortex lesions (Neo-PRh: shaded diamonds, dashed lines) and sham operated controls (Neo-C: open circles, solid line). Graph in [A] illustrates performance of the animals tested at 48 months in the VPC tasks using color stimuli. Graph in [B] illustrates performance of the same animals on the B&W VPC task. Chance is at 50%. *indicates significant group differences ($p<.05$).

Figure 4. Schematic diagram of the Constant Negative Task adapted from Browning and colleagues (2013). During each daily session, monkeys were given a set of 60 unique discrimination problems and chose between a rewarded novel object (S+ shown in gray) and an unrewarded object that was repeated in each daily session (S- shown in black). A 30-s interval separated each discrimination problem.

Figure 5. Average number of Trials [A] and Errors [B] for group Neo-PRH (shaded bars) and sham operated Controls (open bars) to meet the 90% correct learning criterion on the Constant Negative task. Bars represents ±SEM, and *indicates significant group differences ($p<.05$).

Figure 6. Average number of errors plotted across testing session for group Neo-PRH (shaded diamonds) and group Neo-C (open circles) on the Constant Negative task. Bars represent ±SEM.

Figure 7. Average number of Errors for group Neo-PRh (shaded diamonds, dashed line) and group Neo-C (open circles, solid line) to meet the 90% correct criterion for the Constant Negative task versus the Concurrent Discrimination Task. Data for the concurrent discrimination task were generously provided by A. Kazama and J. Bachevalier. Bars represents ±SEM, and *indicates significant group differences ($p<.05$).

Figure 1: Coronal MR images from a representative case (Neo-PRh-5).

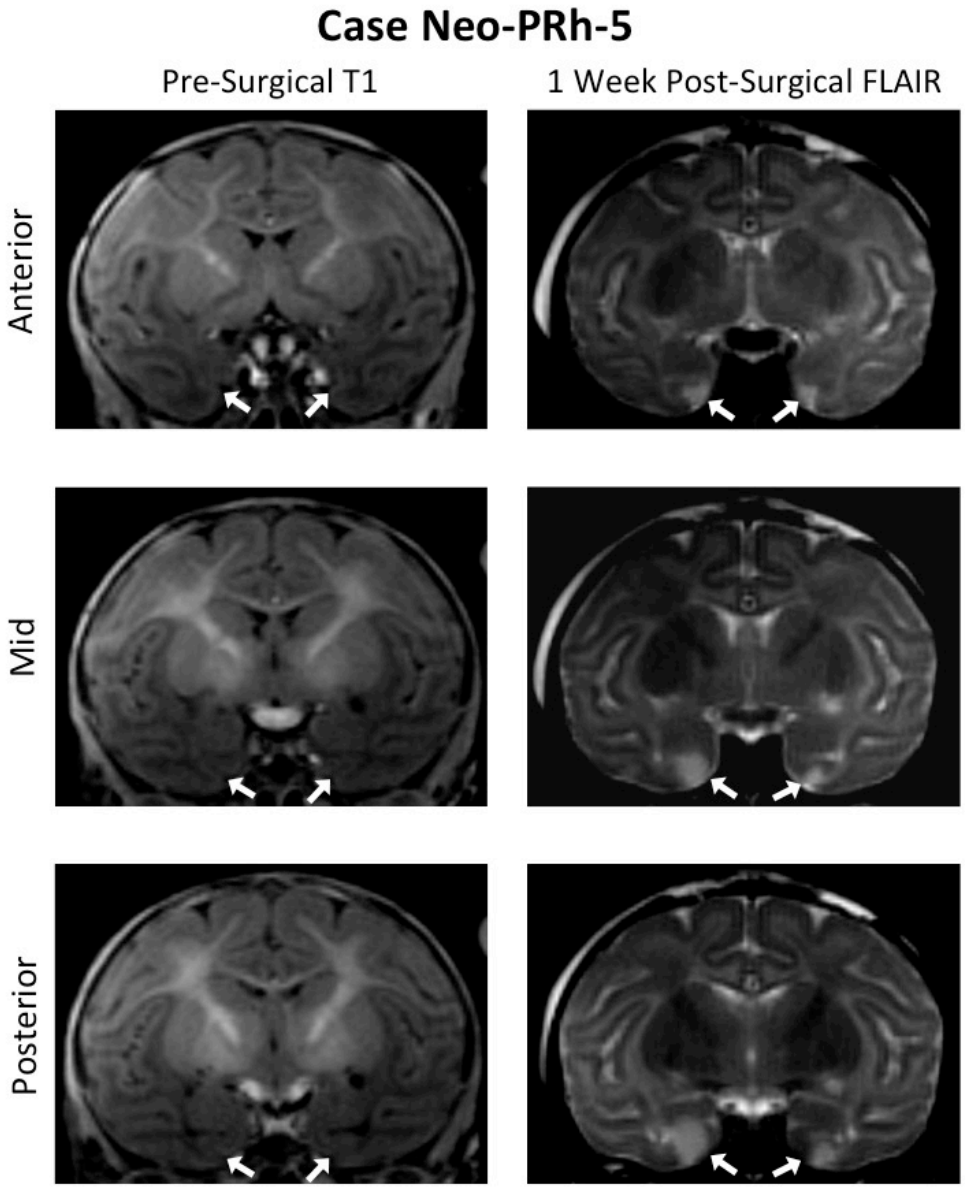


Figure 2: Schematic of B&W Visual Paired Comparison (VPC) Task.

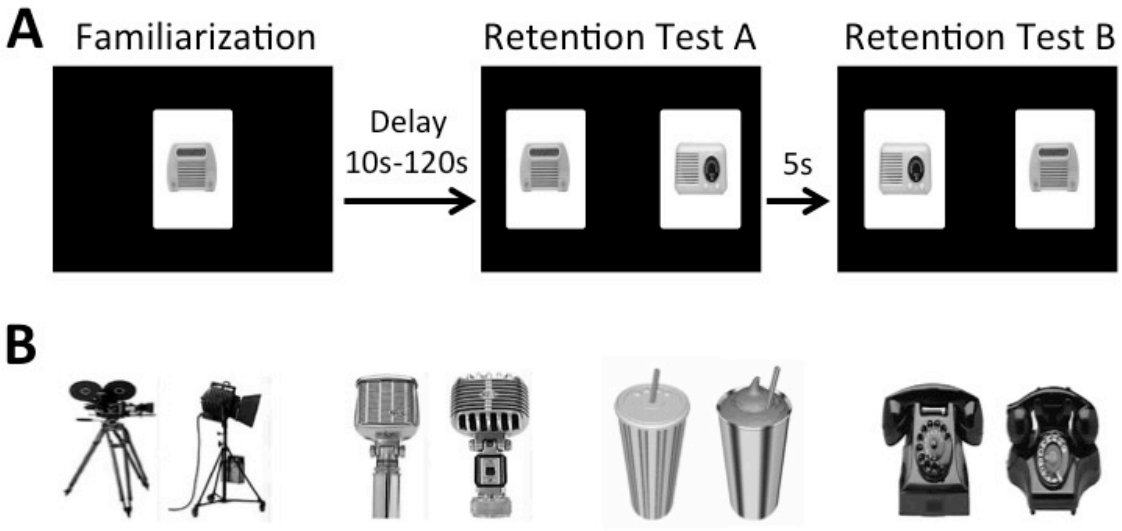


Figure 3: Black and White VPC.

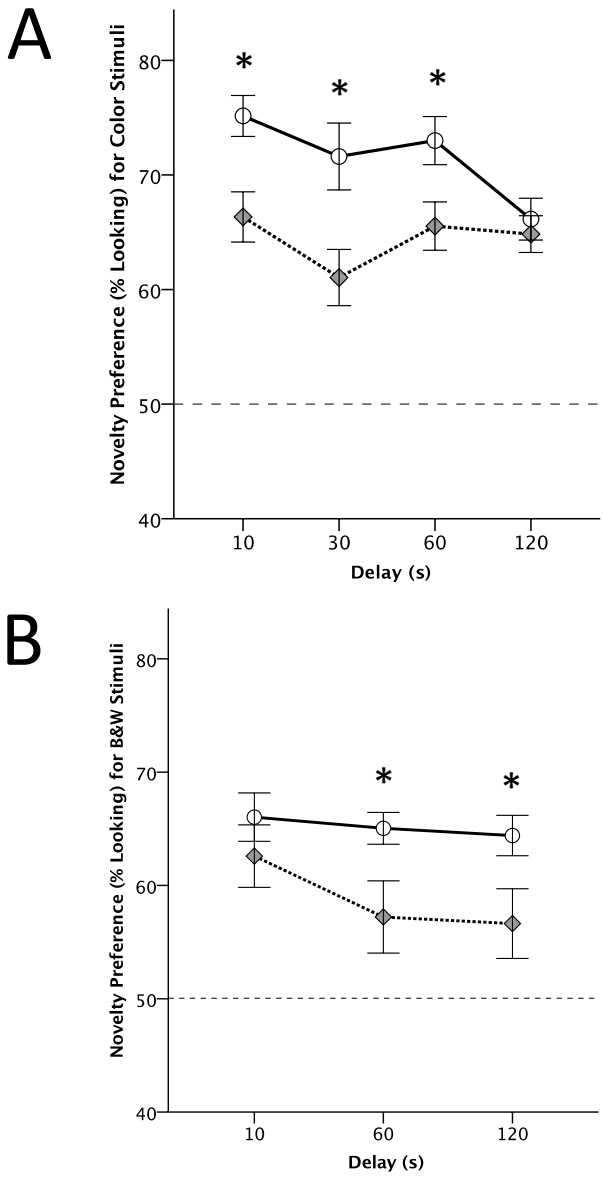


Figure 4: Schematic diagram of the Constant Negative task.

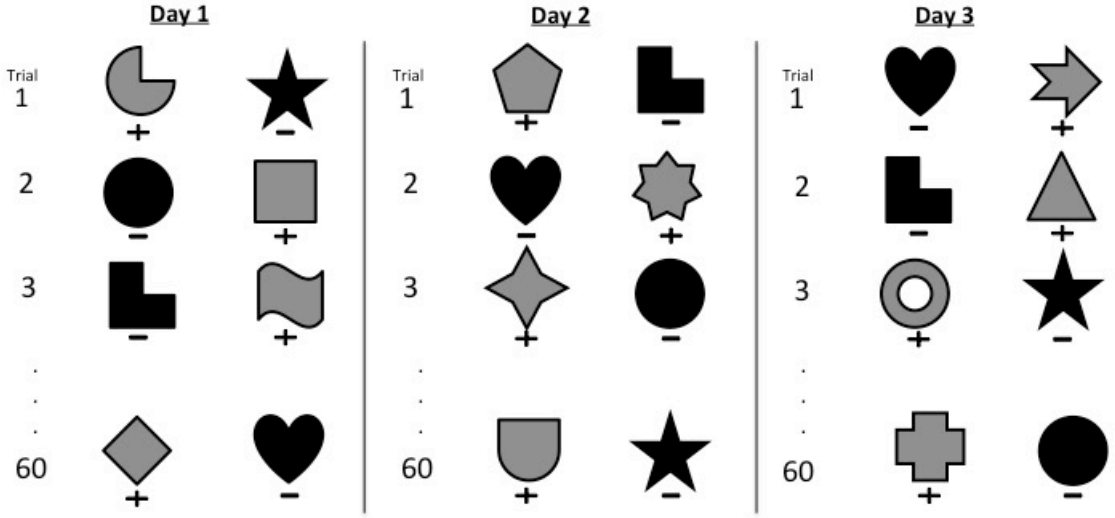
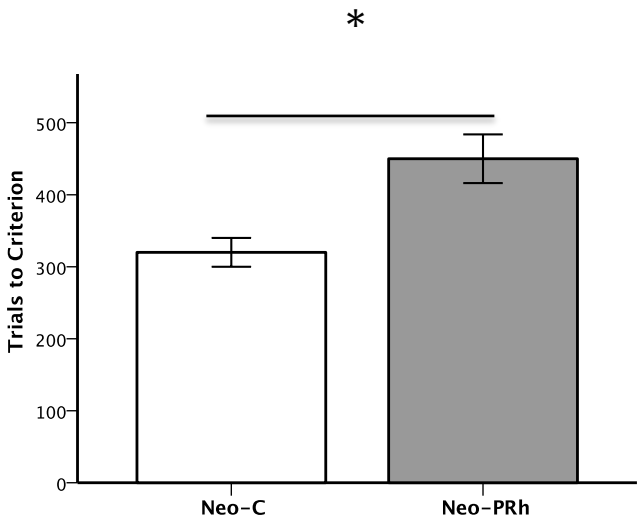


Figure 5: Constant Negative Trials and Errors to Criterion**.**

A



B

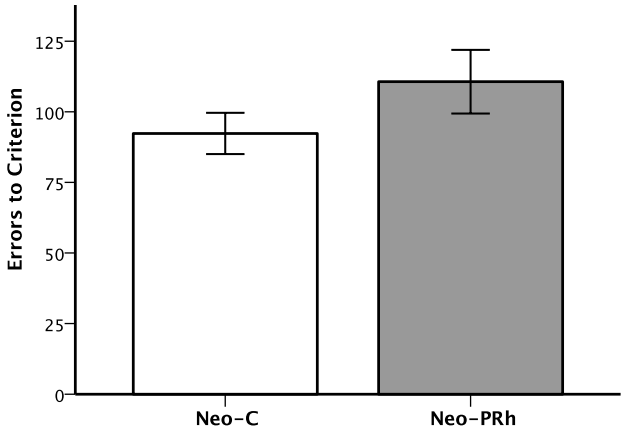


Figure 6: Errors by testing sessions

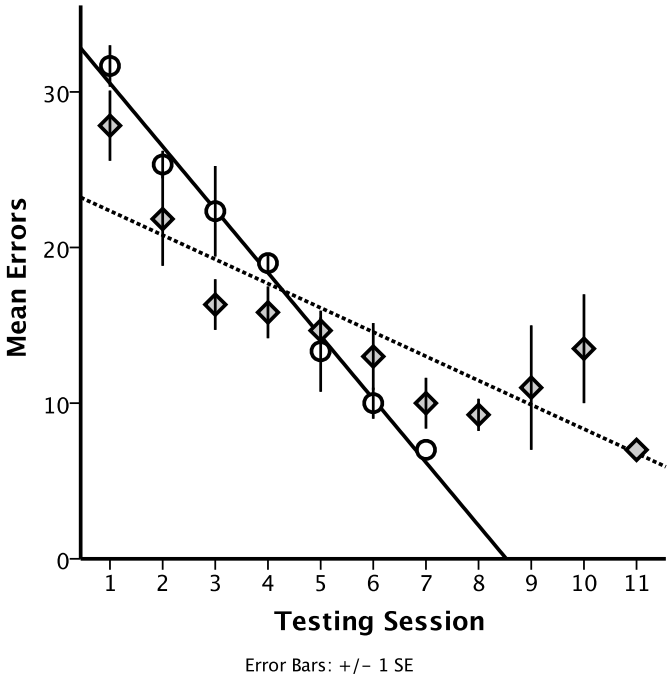


Figure 7: Comparison between Constant Negative and Concurrent Discrimination**.**

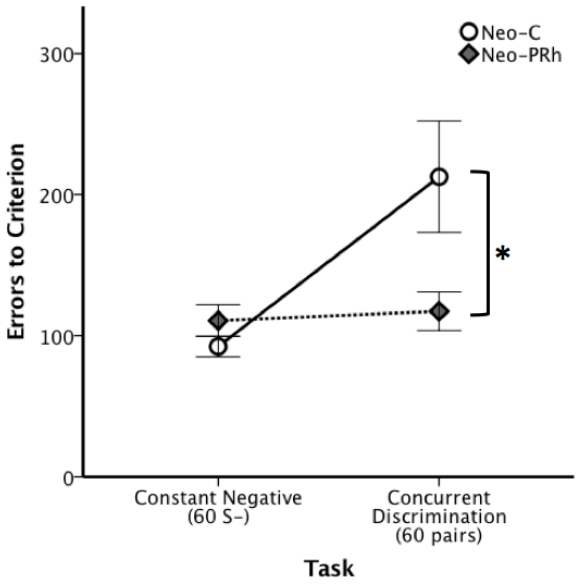


Table 1: Summary of Lesion Extents**.**

Subjects	PRh				ERh				TE			
	L%	R%	X%	W%	L%	R%	X%	W%	L%	R%	X%	W%
Neo-PRh-1	89.76	79.91	83.34	69.04	28.51	2.28	15.39	0.65	4.53	9.70	7.11	0.44
Neo-PRh-2	68.16	70.58	69.37	48.11	17.72	20.65	19.19	3.36	0.14	0.06	0.10	0.00
Neo-PRh-3	65.45	81.02	73.23	53.02	7.72	3.12	5.42	0.24	0.26	3.39	1.82	0.01
Neo-PRh-4	59.40	74.73	67.06	44.39	11.55	17.84	14.69	2.06	0.72	2.62	1.67	0.02
Neo-PRh-5	75.90	66.81	71.35	50.71	38.60	29.86	34.32	11.53	0.72	0.41	0.57	0.00
Neo-PRh-6	74.12	80.31	77.22	59.53	25.34	43.64	34.49	11.06	0.37	2.93	1.65	0.01
Average	*72.13*	*75.06*	*73.60*	*54.13*	*21.57*	*19.57*	*20.57*	*4.87*	*1.12*	*3.19*	*2.15*	*0.08*

Subjects	TH/TF				AMY				HF			
	L%	R%	X%	W%	L%	R%	X%	W%	L%	R%	X%	W%
Neo-PRh-1	0.00	0.00	0.00	0.00	8.24	10.86	9.55	0.89	0.13	2.39	1.26	0.00
Neo-PRh-2	0.00	0.00	0.00	0.00	0.00	2.76	1.38	0.00	0.00	0.00	0.00	0.00
Neo-PRh-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.27	0.14	0.00
Neo-PRh-4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Neo-PRh-5	7.02	3.93	5.47	0.28	0.00	0.00	0.00	0.00	3.37	0.00	1.68	0.00
Neo-PRh-6	0.00	0.00	0.00	0.00	3.78	4.17	3.97	0.16	3.32	0.32	1.77	0.01
Average	*1.17*	*0.66*	*0.91*	*0.05*	*2.00*	*2.96*	*2.48*	*0.18*	*1.12*	*0.50*	*0.81*	*0.00*

Scores are estimates of intended and unintended damage following Neo-PRh lesions for each case. L% = percent damage to left hemisphere; R% = percent damage to right hemisphere; X% = average damage to both hemispheres; W% = weighted damage to both hemispheres (W% = (L% X R%)/100). PRh, perirhinal cortex; ERh, entorhinal cortex, TE, temporal cortical area; TH/TF, parahippocampal cortex; AMY, amygdala; HF, hippocampal formation. Lesion extents from cases Neo-PRh-1 thru Neo-PRh-6 were previously reported by Zeamer et al. (2015).

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Neonatal perirhinal lesions in rhesus macaques alter performance on working memory tasks with high proactive interference

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Abstract

The lateral prefrontal cortex is known for its contribution to working memory (WM) processes in both humans and animals. Yet, recent studies indicate that the prefrontal cortex is part of a broader network of interconnected brain areas involved in WM. Within the medial temporal lobe structures, the perirhinal cortex, which has extensive direct interactions with the lateral and orbital prefrontal cortex, is required to form active/flexible representations of familiar objects. However, its participation in WM processes has not be fully explored. The goal of this study was to assess the effects of neonatal perirhinal lesions on maintenance and monitoring WM processes. As adults, animals with neonatal perirhinal lesions and their matched controls were tested in three object-based (non-spatial) WM tasks that tapped different WM processing domains, e.g. maintenance only (Session-unique Delayed-nonmatching-to Sample, SU-DNMS), and maintenance and monitoring (Object-Self-Order, OBJ-SO; Serial Order Memory Task, SOMT). Neonatal perirhinal lesions transiently impaired the acquisition of SU-DNMS at a short (5s) delay, but not when re-tested with a longer delay (30s). The same neonatal lesions severely impacted acquisition of OBJ-SO task, and the impairment was characterized by a sharp increase in perseverative errors. By contrast, neonatal perirhinal lesion spared the ability to monitor the temporal order of items in WM as measured by the SOMT. Contrary to the SU-DNMS and OBJ-SO, which re-use the same stimuli across trials and thus produce proactive interference, the SOMT uses novel objects on each trial and is devoid of interference. Therefore, the impairment of monkeys with neonatal perirhinal lesions on SU-DNMS and OBJ-SO tasks is likely to be

caused by an inability to solve working memory tasks with high proactive interference. The sparing of performance on the SOMT demonstrates that neonatal perirhinal lesions do not alter working memory processes per se but rather impact processes modulating impulse control and/or behavioral flexibility.

Keywords: excitotoxic lesion, self-ordered task, serial order memory, perseveration, proactive interference

Introduction

Working memory (WM) defines the psychological and neural processes responsible for keeping active a limited set of cognitive representations, and the executive capacity that acts upon those transiently stored representations. In other words, representations of objects, places, ideas, goals, or rules are maintained in WM and flexibly cooperate with process that monitor or manipulate the representations being kept 'in mind.' Domain-specific models of WM have proposed that the lateral prefrontal cortex has a topographical organization according to specific WM processes. Evidence from human functional imaging (Cannon et al., 2005; D'Esposito et al., 1999; Owen et al., 1999; Petrides, 2000), and lesion studies in monkeys (Kowalska et al., 1991; Mishkin & Manning, 1978; Passingham, 1975; Petrides, 1991, 1995), strongly support a distinction between the ventrolateral PFC (vlPFC) associated with maintenance processes and dorsolateral PFC (dlPFC) associated with monitoring/manipulation processes. However, more recent studies suggest that the prefrontal cortex is part of a broader network of interconnected brain areas involved in WM (see for review Constantinidis & Procyk, 2004). Specifically, medial temporal lobe (MTL) structures are also recruited during WM tasks (Davachi & Goldman-Rakic, 2001; Diamond et al., 1989; Kimble & Pribram, 1963; Libby et al., 2012; Petrides, 1991, 1995; Ranganath et al., 2004; Stern et al., 2001). In a recent report, Heuer and Bachevalier (2011) demonstrated that neonatal damage to the hippocampus in monkeys resulted in severe loss of WM-monitoring abilities, but spared WM-maintenance abilities. Given that the only direct inputs of the hippocampus to the PFC target the ventromedial PFC via the fornix, but

not the vlPFC or dlPFC (Cavada, Tejedor, Cruz-Rizzolo, & Reinoso-Suárez, 2000; Croxson et al., 2005), bottom-up information from the hippocampus to the dlPFC will need to be realized via a multisynaptic pathway. Yet, the dlPFC projects back to the posterior hippocampus (Goldman-Rakic, Selemon, & Schwartz, 1984; Morris, Pandya, & Petrides, 1999) providing a potential top-down mechanism regulating hippocampal-dependent WM processes.

Another MTL structure well positioned to play a prominent role in WM processes is the perirhinal cortex (PRh), which has direct reciprocal connections not only with the hippocampus but also with lateral and orbital PFC fields (Lavenex, Suzuki, & Amaral, 2002, 2004; Saunders, Mishkin, & Aggleton, 2005; Suzuki & Amaral, 1994a, 1994b). In addition, electrophysiological and functional imaging studies have reported increased activity in PRh during object-based WM tasks, and PRh neurons of adult macaques are highly activated during WM tasks requiring the temporary maintenance of object representations (i.e. small-set delayed-match-to-sample). Such neuronal changes were not observed in other temporal visual areas, such as area TE (Lehky & Tanaka, 2007). Likewise, 2-deoxyglucose imaging studies indicate increased activity in PRh (but not the entorhinal cortex) during a delayed object alternation task; a task requiring the maintenance and monitoring of information in WM (Davachi & Goldman-Rakic, 2001). Taken together, these results point to a unique contribution of the PRh to performance on tasks that require the active/flexible representation of familiar objects.

Although the critical contribution of the PRh to recognition and stimulus-stimulus association memory has been well documented (Brown & Aggleton, 2001;

Lavenex et al., 2004; Lee et al., 2006; Murray et al., 1993; Warburton & Brown, 2010), its participation in WM processes remains to be fully investigated. In a longitudinal developmental study aimed at tracking the long-term effects of neonatal PRh cortex lesions on memory processes, we recently demonstrated that these early-onset lesions yielded severe recognition memory deficits that emerged in infancy and persisted until adulthood (Weiss & Bachevalier, 2016; Zeamer, Richardson, Weiss, & Bachevalier, 2015). In the present study, we tested whether the same neonatal PRh lesions will result in WM deficits and whether the deficits will encompass both maintenance and monitoring WM processes. As they reached adulthood, animals with neonatal PRh lesions and their controls were successively tested in three object-based working memory tasks previously used to assess the effects of neonatal hippocampal lesions on WM processes (Heuer & Bachevalier, 2011, 2013).

Material and Methods

Subjects

Fifteen adult rhesus macaques (*Macaca mulatta*), 9 females and 6 males, participated in this study. Between postnatal days 10-12, the animals underwent surgery to create bilateral lesions of the perirhinal cortex, or sham operations. Six infant monkeys (3 females, 3 males) were given MRI-guided ibotenic acid injections into perirhinal areas 35 and 36 (Group Neo-PRh), seven monkeys (5 female, 2 male) underwent the same surgical procedures withholding any injections (Group Neo-C), and two additional monkeys (1 female, 1 male) served as un-operated controls. At the time

of this study, all animals were 6-7 years old and housed individually in a room with a 12hour light/dark cycle (7AM/7PM). Monkeys were fed Purina Old World Primate chow (formula 5047) and supplemented with fresh fruit enrichment. During behavioral testing, chow was restricted and the weight of the animals was monitored and maintained at or above 85% of the full feed weight. Water was given ad libitum. One cohort of subjects were born at the YNPRC breeding colony (Lawrenceville, Georgia), and a second cohort were born at the breeding colony of the University of Texas, M.D. Anderson Cancer Center Science Park (Bastrop, TX). At both institutions, all animals received similar rearing and behavioral procedures, including social interactions with age-matched peers and human caregivers as described previously (for detailed description see Goursaud and Bachevalier, 2007; Raper et al., 2013).

All animals had received extensive, but similar, cognitive testing before they participated in this experiment, including tests of incidental recognition memory (visual paired comparison at 1, 6 and 18 months) (Zeamer et al., 2015), oddity learning (3 and 15 months), concurrent discrimination learning with devaluation (48 months), and object and spatial recognition memory (60 months) (Weiss & Bachevalier,2016).

All protocols were approved by the Institutional Animal Care and Use Committee at Emory University in Atlanta, Georgia and conformed to the NIH Guide for the care and use of Laboratory Animals (National Research Council (US), 2011).

Neuroimaging and Surgical Procedures

All neuroimaging and surgical procedures were described in detail by Zeamer et

al. (2015) and are briefly summarized below. To determine injection coordinates prior to surgical procedures and assess lesion extent post-surgery, subjects were given MRIs immediately prior to surgery and 6-8 days post-surgery. At both time points, animals were sedated (10mg/kg of 7:3 Ketamine Hydrochloride, 100mg/ml, and Xylazine, 20mg/ml, administered i.m.) and intubated to allow inhalation of isoflurane (1%-2%, v/v) and maintain an appropriate plane of anesthesia during the duration of the scan. An IV drip (0.45% NaCl and dextrose) was provided for normal hydration and the animal's head was restrained in a stereotaxic apparatus. Vital signs (heart and respiration rates, blood pressure, body temperature and expired CO_2) were constantly monitored during the scan and surgical procedures. The brain was imaged with a 3T Siemens Magnetom Trio system (Siemens Medical Solutions, Malvern, PA at YNPRC) using a 5-cm surface coil and two sets of images were obtained: 1) high-resolution structural images (3D T1-weighted fast spoiled gradient (FSPGR)-echo sequence, TE=2.6ms, TR=10.2ms, 25° flip angle, contiguous 1mm sections, 12cm FOV, 256 x 256 matrix); and 2) Fluid Attenuated Inversion Recovery (FLAIR) images (TE = 140ms, TR = 1000ms, inversion time (TI) = 2200ms, contiguous 3mm sections, 12cm FOV, 256 x 256 matrix; image sequences acquired in 3 series offset 1mm posterior). The pre-surgical T1-weighed images were used to calculate the injection sites and all pre- and post-surgical images were used to estimate the extent of PRh damage as well as damage to adjacent structures.

Following the pre-surgical scans, animals were maintained with Isoflurane gas (1%-2%, v/v, to effect) during the surgical procedures, which were performed under deep anesthesia using aseptic conditions. The scalp was shaved and cleaned with

chlorhexidine diacetate (Nolvasan, Pfizer). A long-lasting local anesthetic, Bupivacaine Hydrochloride (Marcaine 25%, 1.5ml), was injected along the planned midline incision of the scalp, which extended from the occipital to the orbital ridge. After retraction of the galea, bilateral craniotomies (1cm wide x 2.5cm long) were made with an electric drill above the areas to be injected, and bone wax (Ethicon, Inc., Somerville, NJ; 2.5g size) was applied as necessary to prevent bleeding. The dura was opened and injections of 0.4µl ibotenic acid (Biosearch Technologies, Novato, CA, 10mg/ml in PBS, pH 7.4, at a rate of 0.4µl/min) were made 2mm apart along the rostral-caudal length of the perirhinal cortex bilaterally. Sham-operated controls (Neo-C) underwent the same procedures, however once the dura was cut, no injections were made.

The dura, galea, and skin were closed in anatomical layers and the animal was removed from isoflurane, extubated, and closely monitored until complete recovery from anesthesia. Analgesic (acetaminophen, 10mg/kg, p.o.) was given QID for 3 days after surgery. Additionally, animals received dexamethazone sodium phosphate (0.4mg/kg, i.m.) to reduce edema, and Cephazolin (25 mg/kg, i.m.) once a day starting 12h prior to surgery and ending 7 days after to prevent infection.

Lesion Assessment

Histological evaluations are unavailable, as all animals are currently participating in other experiments. Hence, lesion extent was estimated using the MRI images following methods described in details in earlier publications (Nemanic et al., 2002; Málková et al., 2001). Briefly, coronal FLAIR images acquired 1-week post-surgery were

used to examine areas with water hyper-signals (edema) induced by cell death. Areas of hyper-signals seen in each coronal section were drawn onto corresponding coronal sections of a normal 1-week-old rhesus monkey brain (J. Bachevalier, unpublished atlas) using Adobe Photoshop. These images were then imported into Image J® and the surface area of hyper-signals in brain regions of interest (PRh, visual area TE/TEO, entorhinal cortex, parahippocampal cortex, amygdala, and hippocampus) was calculated in pixels^2 and multiplied by image thickness (1mm) to obtain the lesion volume. The percent of damage to each structure was obtained by dividing the volume of the lesion for a given structure by the volume of that same structure in the control atlas and multiplying by 100.

Apparatus and Stimuli

All behavioral tasks were conducted using the Wisconsin General Testing Apparatus (WGTA) located in a dark room with a white-noise generator. Monkeys were transferred from their home cages and positioned in the WGTA facing a tray with 3 recessed food wells (2cm diameter, 1cm deep, spaced 13cm apart). A collection of 1,000 junk objects that differed in size, shape, color, and texture was used. Correct responses were rewarded with preferred food rewards (i.e. mini-marshmallow, jelly bean, M&M etc.)

Session-Unique Delayed Nonmatching-to-Sample (SU-DNMS)

Session-Unique Delayed Nonmatching-to-Sample (SU-DNMS) measured the

maintenance of information in working memory and used training procedures described in Heuer and Bachevalier (2011). For each daily training session, a new pair of objects was selected from a collection of 1,000 junk objects without replacement. Each trial consisted of two phases: sample and choice. During the sample phase, the monkey was presented with a single object covering a reward, followed by a delay of 5s. In the choice phase, two objects, the sample object and the second object, were presented and the monkey was rewarded for selecting the object that was not rewarded during the sample phase. Following a 30s intertrial interval, the same two objects were used for the next trial as well as for all 30 trials of the daily session. The object serving in the sample phase varied on each trial using a pseudorandom sequence. In the first trial, the two objects were novel, but as the daily session progresses, the two stimuli became highly familiar and generated proactive interference. Thus, in SU-DNMS familiarity/novelty judgments cannot be used to guide responses, rather subjects were required to generate responses based on recency memory and inhibit responses based on recognition memory. Learning criterion was set at 90% or better (27 out of 30) in one session, followed by a performance of 80% or better (24 out of 30) in the next training session. Training was discontinued after a maximum of 1,000 trials if criterion was not met. Once subjects met learning criterion at the 5s delay, testing was continued in the same way using a 30s delay and a 30s inter-trial interval. At this longer delay, subjects performed 20 trials per day, again using a novel pair of objects each day, until a learning criterion of 85% averaged over two consecutive testing sessions was achieved, or to a maximum of 500 trials.

The total number of errors (incorrect choices) until meeting criterion at each delay was used as a measure of learning. We also examined how the errors were distributed between the two objects across the daily trials. If errors were distributed equally between the objects, it suggested that the cause of the errors was an impaired ability to maintain information in working memory. On the other hand, if errors were biased towards one object, it instead suggested that the cause of the errors was an impairment of non-mnemonic processes important to support task performance. To test this proposal, we computed an Object Error Distribution Ratio by calculating the absolute value of percent errors made for each object during each daily session minus 50% [# Errors per Object/Total Errors in Session)*100%)-50%)]. These values ranged from 0-50, where 0 represented an equal distribution of errors between the two objects and 50 represented a complete bias towards one of the objects.

Object Self-Ordered Task (OBJ-SO)

This task measured both maintenance and monitoring WM processes, and procedures replicated those described in Heuer and Bachevalier (2011). A set of three new objects, not used in the SU-DNMS task were selected for the OBJ-SO task. During each daily testing session, monkeys chose 3 objects, one at a time, during 3 successive trials. At the start, all three objects were presented covering each of the three food wells with a food reward (Trial 1). Once the monkey made a first choice, the position of the objects on the tray was shuffled and only the two objects unselected in Trial 1 were baited in Trial 2. After the second choice, the positions of the objects were once again

shuffled and only the single remaining (unselected) object in Trials 1 and 2 was baited on Trial 3. The same 3 objects were used in all daily testing sessions and were presented at 10s inter-trial intervals. If, at any time during Trial 2 or 3, the monkey selected an unbaited object, this initial error was scored as a primary error and a correction procedure was initiated. Correction procedures involved reordering the objects and re-presenting them to the monkey until a rewarded object was selected. The number of times the correction procedure was repeated indicated the number of perseverative errors. For analyses, primary and perseverative errors were calculated separately for Trial 2 or Trial 3. Additionally, the percent of errors on Trial 3 that were "repeats" of the errors made on Trial 2 were also tabulated as a measure of impulsive responding.

Learning criterion for the OBJ-SO task was met when subjects scored 85% correct across 10 consecutive daily sessions (3 primary errors or fewer), or testing was discontinued if subjects reached a maximum of 50 daily sessions. Thus, in OBJ-SO monkeys were rewarded for making choices based on the temporal sequence of their own object selections in previous trials of the daily testing session.

Serial Order Memory Task (SOMT)

Similar to the OBJ-SO task, the SOMT assessed both maintenance and monitoring WM processes and was delivered using procedures described by Heuer and Bachevalier (2013). A pool of new objects was selected for each trial of this task from another collection of 1,000 junk objects that differed in size, shape, color, and texture. The objects were divided in 25 bins of 40 objects each and each bin was selected for

testing one at a time until all 25 bins were used before re-using the first bin. Thus, objects only reappeared about once per month. A trial of SOMT consisted of two phases: the sample phase and the test phase. In the sample phase, a list of objects were presented one at a time at 10s intervals and covered the baited center food-well. After displacing the last object of the list and retrieving the food rewards, there was a 10s delay after which the test phase began. In the test phase, two of the objects from the list were selected and covered the lateral food-wells. The monkey was rewarded for displacing the object that occurred earliest in the list. After a 30s inter-trial interval, the next trial began using a new set of objects and a total of 10 trials were given for each daily session.

The monkeys were first trained to criterion using lists of 3 objects. Training progressed in stages: during Stage 1, the test phase paired the first and third objects (1v3), Stage 2 paired the first and second (1v2), and Stage 3 paired the second and third (2v3). The monkey was required to score 80% (8/10) correct during a daily session before moving to the next stage. If the monkey scored 70% (7/10), then that stage was repeated the following session. If the monkey scored 60% or less (6/10), then they were moved back to the previous stage. Once the monkey completed the 3-object version, they moved on to a 4-object version including six stages in which the orders of object pairings in the test phase were as follows: 1v4, 1v3, 1v2, 2v4, 3v4, and 2v3. It is worth noting that only discrimination problems including objects 2v3 required the animals to maintain the order of the objects presented in the list, since with training monkeys could learn that for the other discrimination problems Objects 1 were always rewarded

and Objects 4 were never rewarded. After completing training on the 4-object SOMT, monkeys were tested with probe trials.

Probe trials were administered to assess the ability of the monkeys to track the serial position of objects presented in sequence. This training was identical to the 4-object version described above, except that half of the trials (5 trials) were judgments between 1v4, and the other half (5 trials) were judgments between 2v3. These two trial types were randomized within a daily session so that the monkey could not anticipate which temporal judgments would occur on each trial. Probe trials, therefore, required the monkeys to track ALL of the stimuli in the list. Ten probe trials were administered daily for three consecutive days, resulting in a total of 15 trials of each type. A ratio score was calculated by dividing the total number of correct responses on "inner" pairings (2v3 trials) by the total number of correct responses on "outer" pairings (1v4 trials). A ratio score above or below 1 indicated superior performance on one type of temporal discrimination over another, whereas a score equal to 1 indicated equivalent performance on both trial types.

Data Analyses

Scores of the control animals from the Texas cohort (n = 5) and control animals of the Georgia cohort (n = 4) (see Subjects) were compared across all measures using independent sample t-tests. None reached significance, and so these groups were collapsed in a single control group for all subsequent analyses.

Data obtained from SU-DNMS and OBJ-SO followed a normal distribution, and so

repeated measures ANOVAs were used to compare the scores of the Neo-PRh and Neo-C groups. For SU-DNMS, 2 x 2 ANOVAs (Group x Delay 5s-30s) using Delay as the repeated-factor were performed on the 2 parameters (errors to reach criterion, object error distribution ratio). For OBJ-SO, primary and perseverative Errors were analyzed with a 3-way ANOVA (Group x Error Type x Trial) with repeated measures for the last 2 factors. Finally, independent sample t-tests were used for both tasks to compare the performance of Neo-PRh and Neo-C groups on each measure.

Data from SOMT did not follow a normal distribution, with the exception of the Inner:Outer ratio score. Both nonparametric and parametric analyses were used for all measures. Given the similar pattern of results obtained with both analyses, only the parametric tests will be reported in the "Results" section below. For number of sessions to criterion, a 2 x 2 ANOVA (Group x Object-Pairing) with repeated measures for the second factor was performed. When sphericity was violated, degrees of freedom were adjusted using the Greenhouse-Geisser correction. Comparisons of performance between object pairings were performed for each group separately using a Friedman analysis. Finally, group differences on probe trials (Inner:Outer ratio) were assessed using an independent sample t-test.

Correlations between extent of neonatal PRh lesions or unintended damage to adjacent areas and scores on the three tasks were performed with Pearson correlation. Lastly, for all ANOVAs, effect sizes are reported using eta squared (η^2) and calculated by dividing the sums of squares for the effect of interest by the total sums of squares (Cohen, 1973; Keppel & Wickens, 2004; Levine & Hullett, 2002). For all T-tests, effect

sizes are reported using Cohen's *d* and calculated by dividing the difference between the means of the two groups by the pooled standard deviations (Rosnow & Rosenthal, 1996).

Results

Lesion Assessment

Detailed lesion assessments for all Neo-PRh animals have been published in Zeamer et al. (2015) and percentage of damage to the PRh and adjacent structures is given for each subject of Group Neo-PRh in Table 1. Briefly, all Neo-PRh animals received extensive bilateral damage to the PRh, averaging 73.6% (min=67.1%, max=83.3%). Unintended damage occurred in all cases, mostly in the entorhinal cortex (ERh) (average=20.6%, min=5.4%, max=34.5%), but also minimally in area TE (average=2.5%, min=0.1%, max=7.11%). Four of the six Neo-PRh subjects had negligible damage to the anterior hippocampus (average=0.8%), and three of the six subjects had minimal damage to the amygdala (average=2.5%). The PRh lesion of a representative case (Neo-PRh-4) is illustrated in Figure 1 and two additional cases can be seen in previous publications (see Zeamer et al., 2015, see Figure 2 for case Neo-PRh 3 and Weiss & Bachevalier, under revision, see Figure 1 for case Neo-PRh-2).

SU-DNMS

The numbers of trials and errors to reach the learning criterion at each delay, 5s and 30s, as well as the Object Error Distribution Ratios are reported in Table 2. All

animals reached criterion at both the short and long delays, although animals with Neo-PRh lesions made twice as many errors (Mean: 73 at 5s delay and 34.8 at 30s delay) than controls (Mean: 30.2 at 5s delay and 18.4 at 30s delay; see Figure 2). These group differences were confirmed by a significant group effect on the number of errors to reach criterion [$F(1,13)=5.156$, $p=0.041$, $\eta^2=0.28$]. Planned comparisons revealed that the group difference at the 5s delay was significant [$t(13)=2.207$, $p=0.046$, $d=1.12$,], but not at the 30s delays [$t(13)=-0.811$, $p=0.432$, $d=0.42$]. Furthermore, although both groups improved their performance from the 5s to the 30s delays (see Figure 2), the delay effect and the interaction (Group x Delay) were not reliable [$F(1,13)=2.803$, $p=0.118$, $\eta^2=0.14$; $F(1,13)=0.783$, $p=0.392$, $\eta^2=0.05$], indicating that the magnitude of improvement was similar for both groups.

The Object Error Distribution Ratio (Table 2) was also higher in animals with Neo-PRh lesions than controls at both delays, indicating a tendency to preferentially select one object over the other [$F(1,13)=3.782$, $p=0.075$, $\eta^2=0.23$]. Neither the delay effect nor the interactions between the two factors reached significance [$F(1,13)=0.100$, $p=0.756$, $\eta^2=0.01$ and $F(1,13)=0.150$, $p=0.705$, $\eta^2=0.01$, respectively]. Yet, planned comparisons indicated that the group difference was significant at the 5s delay but not at the 30s delay [$t(13)=2.561$, $p=0.024$, $d=1.42$ and $t(13)=1.143$, $p=0.273$, $d=0.61$, respectively].

Additionally, errors made during the first block of 10 trials and last bock of 10 trials in each daily session of the SU-DNMS task were tallied separately to determine if the monkeys tended to make more errors at the end of the session. A Group x Trial-

Block (first-last) ANOVA with repeated measure for the second factor revealed a significant main effect of Group at the 5s delay [F(1,13)=5.107, p=0.042, η2=0.282], but not at the 30s delay [F(1,13)=0.754, p=0.401, η2=0.055] and a significant effect of Trial-Block at the 5s delay [F(1, 13)=5.084, p=0.042, η^2=0.272] but not at the 30s delay F(1,13)=3.672, p=0.078, η^2=0.218]. None of the interactions were significant [5s: F(1,13)=0.640, p=0.438, η^2=0.034; 30s: F(1,13)=0.142, p=0.712, η^2=0.008]. Thus, both groups of monkeys tended to make more errors on the last 10 trials than on the first 10 trials at 5s delay, but not at 30s delay.

OBJ-SO

Control animals reached criterion in an average of 12.7 testing days. In contrast, all but one of the 6 animals with Neo-PRh cortex lesions (Neo-PRh-5) failed to reach criterion within the limit of testing (50 testing days), resulting in an averaged group performance of 43 [t(13)=-3.454, p=0.004, *d*=1.81; see Table 1]. As shown in Figure 3 (A and B), this learning impairment was also reflected by a greater number of primary and perseverative errors on Trial 2 and Trial 3 made by Neo-PRh animals as compared to the Neo-C animals [Primary errors: t(13)=-3.444, p=0.004, *d*=1.68 and t(13)=-2.647, p=0.020, *d*=1.41 for Trial 2 and Trial 3, respectively; Perseverative errors: t(5.736)=-2.836, p=0.031, *d*=1.61 and t(13)=-2.901, p=0.012, *d*=1.50, for Trial 2 and Trial 3 respectively]. The 3-way ANOVA (Group x Error types x Trials) revealed significant main effects of Group [F(1,13)=9.597, p=0.008, η^2=0.42] and Trial [F(1,13)=22.716, p<0.001, η^2=0.55], but not of Error Type [F(1,13)=2.819, p=0.117, η^2=0.15]. The 3-way interaction also

reached significance [$F(1,13)=10.545$, $p=0.006$, $\eta^2=0.21$]. Thus, although both groups made more primary and perseverative errors on Trial 3 than on Trial 2, Group Neo-C had a similar increase in primary and perseverative errors across trials. By contrast, for Group PRh, the increase in perseverative errors from Trial 2 to Trial 3 was greater in magnitude than the increase in primary errors [Group x Trial interaction: $F(1,13)=7.217$, $p=0.019$, $\eta^2=0.13$ and $F(1,13)=2.172$, $p=0.164$, $\eta^2=0.07$, for Perseverative and Primary Errors, respectively].

Finally, to determine whether the increase of errors in animals with Neo-PRh lesions was due to impulsive reactivity, we assessed the animals' tendency to select in Trial 3 the same incorrect object they selected in Trial 2. The percent of errors on Trial 3 that repeated the errors on Trial 2 did not significantly differ between groups [$t(13)=-0.435$, $p=0.671$, $d=0.24$].

SOMT

The numbers of sessions to reach criterion at each stage of object pairings on the 3-Object and 4-Object versions of this task are reported in Table 3. All monkeys acquired the task within the maximum number of sessions (20 per stage). On the 3-Object version, the effects of group (Neo-C vs Neo-PRh), Object-Pairing stages (i.e. 1v3, 1v2, 2v3) and their interaction did not reach significance [$F(1,12)=0.827$, $p=0.381$, $\eta^2=0.064$; $F(1.230, 14.758)=3.312$, $p=0.083$, $\eta^2=0.216$; $F(1.230, 14.758)=0.023$, $p=0.920$, $\eta^2=0.002$, respectively]. A similar pattern emerged on the 4-Object version [Group: $F(1,12)=3.197$, $p=0.099$, $\eta^2=0.210$]; 6 Object-Paring stages: $F(2.503, 30.040)=0.490$, $p=0.659$, $\eta^2=0.036$;

Group x Object-Pairing interaction: F(2.503, 30.040)=1.007, p=0.392, η^2=0.075].Therefore, both groups performed similarly on the 3-Object and 4-Object versions of the task.

Results of the probe trials are reported in Table 3. The Inner:Outer ratio scores of the Neo-PRh group averaged 0.84, indicating slightly better performance on 1v4 pairings that 2v3 pairings. The Neo-C group averaged 0.97, indicating approximately equal performance on both pairings. However, the group difference was not significant [t(11)=-1.375, p=0.197, *d*=0.76].

Correlations

Finally, none of the correlations between the average extent bilateral of PRh damage and scores on each of the 3 working memory tasks reached significance (all *p*s > .05), indicating that greater extent of lesions was not related to performance on any of the tasks (see Supplemental Materials for details).

Comparisons with neonatal hippocampal lesions

To investigate how pattern of deficits after the Neo-PRh lesions contrast with those previously reported after neonatal hippocampal (Neo-H) lesions, scores of Neo-PRh and Neo-C on the three working memory tasks were compared to those obtained by the Neo-H groups (Heuer & Bachevalier, 2011, 2013). As shown in Table 2, Neo-H lesions appear to affect SU-DNMS acquisition (50 and 16 errors for 5s and 30s, respectively) to a smaller degree than Neo-PRh lesions (73 and 35 errors for 5s and 30s

respectively). However, differences between the 3 groups did not reach significance [5s errors: $F(2,20)=1.262$, $p=0.307$, $\eta^2=0.123$; 30s errors: $F(2,20)=0.574$, $p=0.573$, $\eta^2=0.060$]. In contrast, the Neo-PRh group was equally impaired in learning the OBJ-SO task as the Neo-H group (see Table 2), both groups averaging 43 and 44 sessions to reach criterion, respectively, as compared to 13 sessions for the controls, [$F(2,20)=7.164$, $p=0.005$, $\eta^2=0.443$; Neo-PRh vs Neo-H: $t(18)=0.130$, $p=0.898$, $d= 0.070$; Neo-PRh vs Neo-C: $t(18)=3.236$, $p=0.005$, $d=1.810$; Neo-H vs Neo-C: $t(18)=-3.094$, $p=0.006$, $d=1.568$]. Finally, comparisons between the effects of Neo-H lesions and Neo-PRh lesions on the SOMT (Table 3) indicated that the Neo-H group required more sessions (5 sessions) to complete the 2v3 phase of the 4-Object version than the Neo-PRh group (3 sessions) or controls (1 session) [$F(2,19)=5.336$, $p=0.016$, $\eta^2=0.386$; Neo-PRh vs Neo-H: $t(17)=-2.026$, $p=0.059$, $d=1.025$; Neo-PRh vs Neo-C: $t(17)=1.083$, $p=0.294$, $d=0.537$; Neo-H vs Neo-C: $t(17)=-3.249$, $p=0.005$, $d=2.114$]. This impairment of temporal order memory for the inner items of a list by the Neo-H group was also apparent in Probe trials, where Neo-H monkeys had lower Inner:Outer ratios (0.68) than the Neo-PRh monkeys (0.84) or Controls (0.97) [$F(2,18)=5.350$, $p=0.017$, $\eta^2=0.401$; Neo-PRh vs Neo-H: $t(16)=1.870$, $p=0.080$, $d=1.038$; Neo-PRh vs Neo-C: $t(16)=-1.324$, $p=0.204$, $d=0.757$; Neo-H vs Neo-C: $t(16)=-3.265$, $p=0.005$, $d=1.806$].

Discussion

This study investigated the effects of neonatal PRh-lesions on WM processes when animals reached adulthood. The results indicate that neonatal PRh-lesions slightly,

but only transiently, impaired WM maintenance processes measured by the SU-DNMS task and impaired WM maintenance/monitoring processes measured by the OBJ-SO task. In contrast to both SU-DNMS and OBJ-SO tasks that generated high proactive interference, performance on the SOMT that was devoid of proactive interference was not altered by the neonatal PRh lesions. The results suggest that neonatal PRh lesions may impact the ability to resolve proactive interference and/or inhibit perseverative responding rather than affecting working memory processes per se. These findings will be discussed in turn.

Maintenance

Monkeys with Neo-PRh lesions initially learned SU-DNMS more slowly than controls. However, the mild impairment at the short delay was not evident with further training at the longer delay of 30s. The same groups of animals were tested on several other memory tasks from infancy through adulthood, and their performance on these tasks can help us reject several interpretations of the transient impairment in the SU-DNMS task. For example, animals with neonatal PRh lesions did not differ from controls in learning a trial-unique delayed nonmatching task indicating no significant impact of the Neo-PRh lesions on perceptual abilities, formation of object representation, learning reward contingencies, or motivation to perform a task (Weiss & Bachevalier, 2016). Furthermore, the impairment at the 5s of the SU-DNMS could not be explained by an inability to maintain object representation across the short delay, given the normal performance at the longer delay of 30s. However, one distinct feature of the SU-DNMS

task that has not been addressed with prior memory tasks given to these groups of animals, but that could be relevant to their impairment in the SU-DNMS, is the increased interference encountered by the animals while responding to successive trials. Indeed, in contrast to all other memory tasks previously performed by the animals, SU-DNMS uses the same two stimuli on every trial of a daily session, generating increased proactive interference as the animals progressed through the task. Thus, the learning impairment observed in animals with Neo-PRh lesions at the 5s delay could be the result of difficulties learning to resolve or inhibit interference. Interestingly, the mild and transitory impairment of the Neo-PRh subjects during the SU-DNMS task is reminiscent to that reported earlier by Eacott and colleagues after rhinal (perirhinal and entorhinal) cortex lesions in adulthood (Eacott, Gaffan, & Murray, 1994). In this latter study, adult monkeys with rhinal lesions were tested in a matching-to-sample task using 4 stimuli and showed transient impairment especially at the shortest delays used and not at the longer delays, and then performed normally when re-tested with only 2 stimuli. This similar pattern of transient deficits after the early-onset and late-onset lesions suggests very little recovery of SU-DNMS performance after the early-onset PRh lesions.

A large body of work has already demonstrated that the hippocampus may be critical to reduce proactive interference (Butterly et al., 2012; Shapiro and Olton, 1994; Aggleton et al., 1986; Bachevalier et al., 2013). Given that the majority of sensory inputs reaching the hippocampus are relayed through the perirhinal cortex, the Neo-PRh lesions could have disconnected the hippocampus from receiving this flow of

information and yielded decreased resistance to interference. However, this explanation seems implausible given that direct damage to the hippocampus does not impair performance on the SU-DNMS (Heuer & Bachevalier, 2011). An alternative explanation may relate to the important interconnections of the perirhinal cortex with the ventrolateral PFC (vlPFC) and orbital frontal cortex (OFC) (Petrides & Pandya, 2002; Lavenex, Suzuki, & Amaral, 2002). Both vlPFC and OFC lesions in adult monkeys yield deficits in rule-learning that were attributed to perseverative interference generated from competition between well-established responses (Mishkin & Manning, 1978; Butter, 1969; Passingham, 1975; Baxter, Browning, & Mitchell, 2008; Baxter et al., 2009; Dias, Robbins, & Roberts, 1996; Meunier, Bachevalier, & Mishkin, 1997). Furthermore, like performance of Neo-PRh monkeys, monkeys with vlPFC lesions require more trials than controls to acquire the DNMS rule and tend to make perseverative errors, but after learning the task, they perform normally on subsequent tests with longer delays (Kowalska et al., 1991). Monkeys with OFC lesions are similarly slow to acquire the DNMS rule, yet their deficit is not overcome with additional training (Meunier, Bachevalier, & Mishkin, 1997). Thus, the deficit in learning the SU-DNMS at short delay may have resulted from a disconnection of the vlPFC from the PRh, preventing vlPFC from accessing object-representations generated by PRh. Yet, the learning deficit in the SU-DNMS after the neonatal PRh lesions was only transitory as was the learning deficit following vlPFC lesions. This improvement in performance suggests that with further training, animals with such lesions can overcome or suppress their perseverative habits, presumably, by developing alternate strategies supported by other PFC areas, such as

the OFC. A recent study investigating the effects of neonatal lesions to the vlPFC and OFC separately or in combination demonstrated that, in the absence of a functional vlPFC in infancy, the OFC can take over and support learning skills (Malkova, Alvarado, & Bachevalier, 2014).

Monitoring

In comparison to the transient impairment on the WM maintenance task, SU-DNMS, the same neonatal PRh lesions severely impacted acquisition of the OBJ-SO task in all but one of the Neo-PRh monkeys. Furthermore, the source of errors during OBJ-SO acquisition differed between the Neo-PRh and Neo-C groups. The Neo-PRh group made more primary errors than the controls, but the increase in primary errors from Trial 2 to Trial 3 was similar for both groups. Furthermore, although the Neo-PRh group made also more perseverative errors than controls, the increase in perseverative errors from Trial 2 to Trial 3 was greater in magnitude for animals with Neo-PRh lesions than for controls. This pattern of results indicates that monkeys with neonatal PRh lesions may be unable to monitor the order of self-generated responses. Alternatively, like the mild learning impairment reported above for the SU-DNMS task, the inability of animals with Neo-PRh lesions to solve the OBJ-SO task could also be due to inability to suppress interference. The OBJ-SO task uses the same three stimuli from trial to trial, and across all daily sessions, resulting in high levels of interference. Thus, as reported above for the SU-DNMS, the severe impairment on the OBJ-SO task after Neo-PRh lesions could be due either to an inability to monitor information in WM and/or to an inability to resolve

interference.

To distinguish between these alternative interpretations, the animals were tested in the SOMT, a WM task that requires the ability to monitor the sequence of object presentations but uses novel objects in each trial. In the SOMT, use of trial-unique stimuli was intended to minimize the impact of interference, and so performance should depend only on the ability to monitor the temporal order of stimuli. Neo-PRh monkeys acquired the SOMT rules similarly to controls, requiring approximately the same number of sessions at each learning stage. During Probe trials, Neo-PRh and Neo-C monkeys made similar numbers of correct choices for temporal judgments between Object 1 and Object 4 as they did for temporal judgments between Object 2 and Object 3, resulting in roughly equivalent Inner:Outer Ratio scores. Thus, measured with SOMT, neonatal PRh lesion appears to spare the ability to monitor items in WM. Therefore, the severe impairments of the same monkeys in OBJ-SO are likely to be caused by impairment in cognitive processes other than WM. Indeed, the increase in perseverative errors found in animals with Neo-PRh lesions while performing WM tasks with high proactive interference may have instead been caused by a lack of impulse control and/or impaired behavioral flexibility.

Comparison with the neonatal hippocampal lesions (Neo-H)

The pattern of deficits in the three working memory tasks after the Neo-PRh lesions contrasted with those reported after the Neo-H lesions (Heuer & Bachevalier, 2011; 2013). Thus, unlike Neo-PRh lesions, Neo-H lesions did not impact the ability to

maintain information in memory but resulted in severe impairment in both tasks measuring monitoring WM processes. Taken together, these data indicate that the perirhinal cortex and the hippocampus play different roles in supporting the development of WM processes; i.e. the hippocampus supporting monitoring WM processes whereas the perirhinal resolving proactive interference.

Conclusions

The present results suggest that the perirhinal cortex may be particularly important to resolve interference. Yet, it is not clear whether the deficits resulted from direct damage to the PRh or from downstream effects of the neonatal PRh lesions on the normal maturation of other neural structures, especially those with protracted anatomical and functional development, such as the PFC (Fuster, 2002; Conklin et al., 2007; Perlman et al., 2015; Overman et al., 2004; Kolb et al., 2010). Developmental studies in rodents (Tseng et al., 2009) and monkeys (Chlan-Fourney et al., 2000; Bertolino et al., 1997; Meng et al., 2013) have already demonstrated significant morphological and neurochemical changes in the lateral PFC as a result of early damage to the MTL structures. Given that the lateral PFC is critical for performance on the WM tasks, the WM deficits after the neonatal PRh lesions may have resulted from maldevelopment of the PFC following disruption of inputs it receives from the PRh rather than damage to PRh per se. Disentangling these alternative interpretations will require the replication of the current experiments in a group of monkeys that will have received the same PRh lesions in adulthood.

Table 1. Extent of neonatal perirhinal lesions

Subjects	PRh				ERh			
	L%	R%	X%	W%	L%	R%	X%	W%
Neo-PRh-1	89.76	79.91	83.34	69.04	28.51	2.28	15.39	0.65
Neo-PRh-2	68.16	70.58	69.37	48.11	17.72	20.65	19.19	3.36
Neo-PRh-3	65.45	81.02	73.23	53.02	7.72	3.12	5.42	0.24
Neo-PRh-4	59.40	74.73	67.06	44.39	11.55	17.84	14.69	2.06
Neo-PRh-5	75.90	66.81	71.35	50.71	38.60	29.86	34.32	11.53
Neo-PRh-6	74.12	80.31	77.22	59.53	25.34	43.64	34.49	11.06
Average	*72.13*	*75.06*	*73.60*	*54.13*	*21.57*	*19.57*	*20.57*	*4.87*

L% = percent damage to left hemisphere; R% = percent damage to right hemisphere; X% = average damage to both hemispheres; W% = weighted damage to both hemispheres (W% = (L% X R%)/100). PRh, perirhinal cortex; ERh, entorhinal cortex. Lesion extents from cases Neo-PRh-1 thru Neo-PRh-6 were previously reported in Zeamer et al. (2015).

Table 2: Performance on the SU-DNMS and Obj-SO tasks

Groups	SU-DNMS						OBJ-SO				
	Trials to Criterion		Errors to Criterion		Object Error Distribution Ratio		Sessions to Criterion	Primary Errors		Perseverative Errors	
	5s	30s	5s	30s	5s	30s		Trial 2	Trial 3	Trial 2	Trial 3
Neo-PRh											
Neo-PRh-1	360	0	106	0	22.6	0.0	50	23	33	8	57
Neo-PRh-2	90	360	30	110	12.8	24.6	50	24	36	11	53
Neo-PRh-3	420	160	102	52	25.8	32.9	50	16	26	4	35
Neo-PRh-4	480	60	129	12	20.1	8.9	50	16	31	7	59
Neo-PRh-5	180	80	43	20	25.8	25.4	8	1	4	0	2
Neo-PRh-6	90	60	28	15	15.9	18.3	50	11	32	3	64
Average	*270.0*	*120.0*	*73.0*	*34.8*	*20.5*	*18.4*	*43.0*	*15.2*	*27.0*	*5.50*	*45.00*
Neo-C											
Neo-C-1	0	0	0	0	0.0	0.0	0	0	0	0	0
Neo-C-2	-	-	-	-	-	-	-	-	-	-	-
Neo-C-3	150	320	35	114	17.5	27.2	6	2	8	0	16
Neo-C-4	240	80	68	16	9.5	22.9	11	2	5	0	13
Neo-C-5	120	0	26	0	18.5	0.0	5	4	3	1	0
Neo-C-6	0	0	0	0	0.0	0.0	1	1	0	0	0
Neo-C-7	300	0	71	0	14.9	0.0	26	6	15	1	17
Neo-C-8	-	-	-	-	-	-	-	-	-	-	-
Neo-C-9	0	0	0	0	0.0	0.0	15	7	13	1	4
Neo-C-10	270	60	66	18	18.1	23.6	50	13	41	4	62
Neo-C-11	30	80	6	18	16.7	23.3	0	0	0	0	0
Average	*123.3*	*60.0*	*30.2*	*18.4*	*10.6*	*10.8*	*12.7*	*3.9*	*9.4*	*0.8*	*12.4*
Neo-H											
Neo-H-1	0	220	0	55	0	15.2	50	8	27	1	28
Neo-H-2	30	40	4	11	25.0	33.3	50	13	33	11	52
Neo-H-3	570	40	190	17	13.2	14.6	0	0	0	0	0
Neo-H-4	60	20	9	10	35.7	10.0	50	15	28	3	39
Neo-H-5	330	0	91	0	16.8	0.0	50	17	32	3	39
Neo-H-6	30	0	5	0	10.0	7.1	50	8	26	2	34
Average	*170.0*	*53.3*	*49.8*	*15.5*	*16.8*	*13.4*	*41.7*	*10.2*	*24.3*	*3.3*	*32.0*

For Session Unique Delayed Non-Match to Sample (SU-DNMS), scores are number of trials and errors to criterion and the error distribution ratio at each delay. For the Object Self-Ordered task (OBJ-SO), scores are number of sessions and errors to criterion. Neo-C-2 and Neo-C-8 were not tested on SU-DNMS or OBJ-SO. Data from Neo-C-1 thru Neo-C-6 and Neo-C-11 previously reported in Heuer and Bachevalier (2011). Data Neo-H-1 thru Neo-H-6 used for comparison in section 3.6 and also reported in Heuer and Bachevalier (2011).

Table 3: Performance on the SOMT task

	SOMT 3-Object			SOMT 4-Object						*SOMT Probe*
Groups	1v3	1v2	2v3	1v4	1v3	1v2	2v4	3v4	2v3	Inner:Outer Ratio
Neo-PRh										
Neo-PRh-1	2	7	1	3	1	1	2	3	1	0.62
Neo-PRh-2	3	3	1	3	3	2	1	2	6	0.71
Neo-PRh-3	2	5	1	1	2	1	1	1	2	0.83
Neo-PRh-4	1	4	7	5	2	5	2	1	1	1.08
Neo-PRh-5	1	11	3	2	5	1	1	1	6	1.00
Neo-PRh-6	2	1	4	1	1	1	3	8	1	0.79
Average	*1.8*	*5.2*	*2.8*	*2.5*	*2.3*	*1.8*	*1.7*	*2.7*	*2.8*	*0.84*
Neo-C										
Neo-C-1	1	3	1	2	2	9	1	1	1	1.08
Neo-C-2	-	-	-	-	-	-	-	-	-	-
Neo-C-3	1	2	3	1	1	3	1	1	1	0.93
Neo-C-4	1	18	1	1	2	2	2	1	1	1.00
Neo-C-5	1	2	2	1	1	3	1	1	1	1.20
Neo-C-6	1	1	3	1	2	3	1	1	1	1.00
Neo-C-7	2	1	1	2	3	2	1	2	4	0.87
Neo-C-8	-	-	-	-	-	-	-	-	-	-
Neo-C-9	1	2	4	2	2	1	1	1	4	0.69
Neo-C-10	-	-	-	-	-	-	-	-	-	-
Neo-C-11	2	4	3	1	1	1	3	4	1	-
Average	*1.1*	*4.1*	*2.1*	*1.4*	*1.9*	*3.3*	*1.1*	*1.1*	*1.9*	*0.97*
Neo-H										
Neo-H-1	1	3	2	1	1	1	1	1	8	0.54
Neo-H-2	1	2	4	1	1	7	1	6	5	0.62
Neo-H-3	1	10	2	1	1	5	2	1	5	1.00
Neo-H-4	2	9	1	1	1	1	1	1	3	0.53
Neo-H-5	1	7	1	1	2	2	2	3	4	0.71
Neo-H-6	1	1	1	1	1	1	1	2	5	0.50
Average	*1.2*	*5.3*	*1.8*	*1.0*	*1.2*	*2.8*	*1.3*	*2.3*	*5.0*	*0.65*

Scores are the numbers of sessions to criterion for each of the object pairings in the 3-objects and 4-objects version of the Serial Order Memory Task (SOMT). Probe ratio are correct choices for "inner" (2v3) problems over correct choices for "outer" (1v4) problems. Neo-C-2, Neo-C-8 and Neo-C-10 were not tested on the SOMT, and Neo-C-11 was not given the SOMT Probe trials. Data from Neo-C-1 thru Neo-C-6 previously reported in Heuer and Bachevalier (2013). Data from animals Neo-H-1 thru Neo-H-6 used for comparison in section 3.6 and also reported in Heuer and Bachevalier (2013).

Figure legends

Figure 1: Coronal MRI from a representative case (Neo-PRh-4). Pre-surgical structural T1-weighted images at three rostro-caudal levels through the perirhinal cortex (left column). Post-surgical FLAIR images (right column) at the same rostro-caudal levels show hypersignals (whiter areas) that are indicative of edema and cell damage. Arrows point to the rhinal sulcus on the left and to hypersignals on the right.

Figure 2: Session-Unique DNMS Performance. Average number (± SEM) of errors to reach criterion on Session-Unique DNMS at delays of 5s and 30s for animals with neonatal perirhinal lesions (filled bars) and controls (open bars).

Figure 3: Object Self-Ordered Task Performance. Average number (± SEM) of primary errors (A) and perseverative errors (B) to criterion on the object self-ordered task (Obj-SO) at delays of 5s and 30s for animals with neonatal perirhinal lesions (filled bars) and controls (open bars).

Figure 1. Coronal MRI from a representative case (Neo-PRh-4).

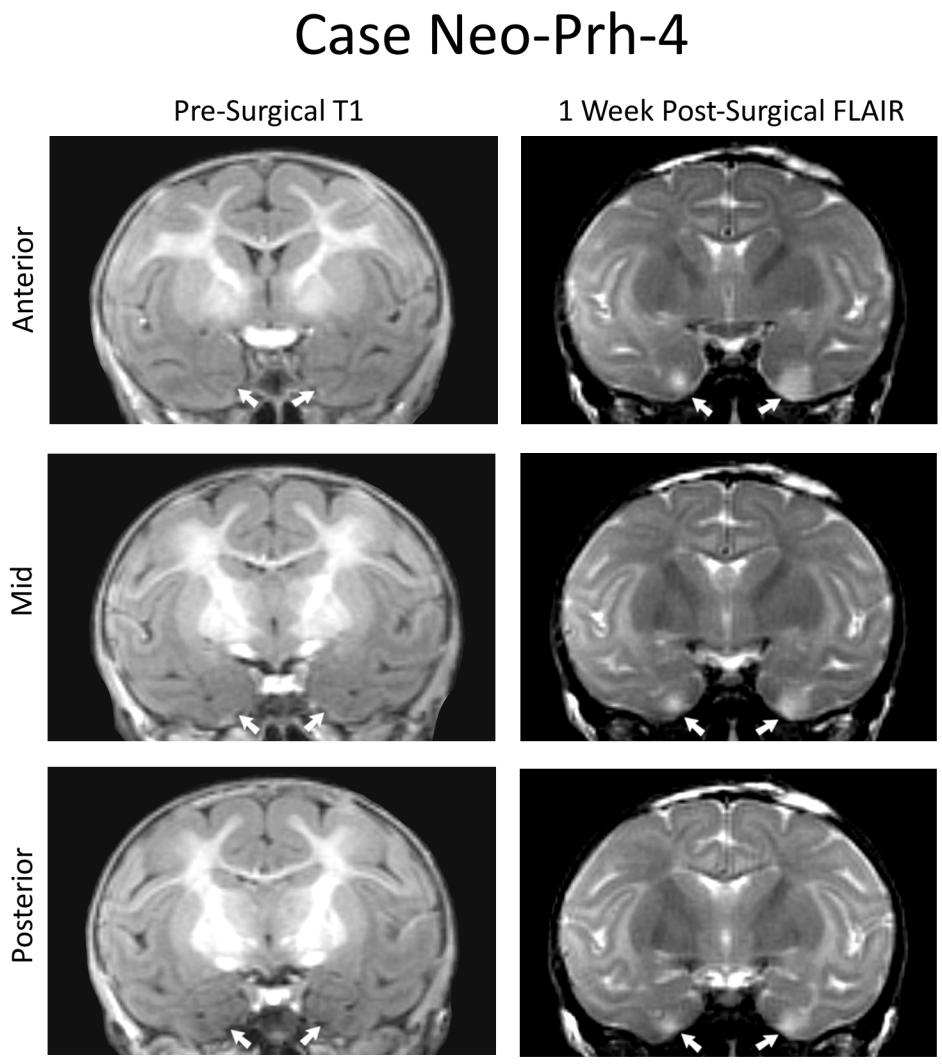


Figure 2. Session-Unique DNMS Performance.

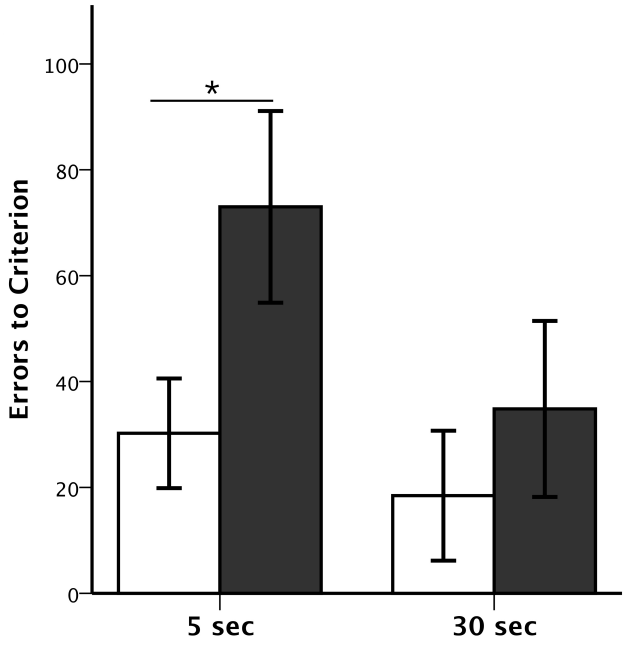
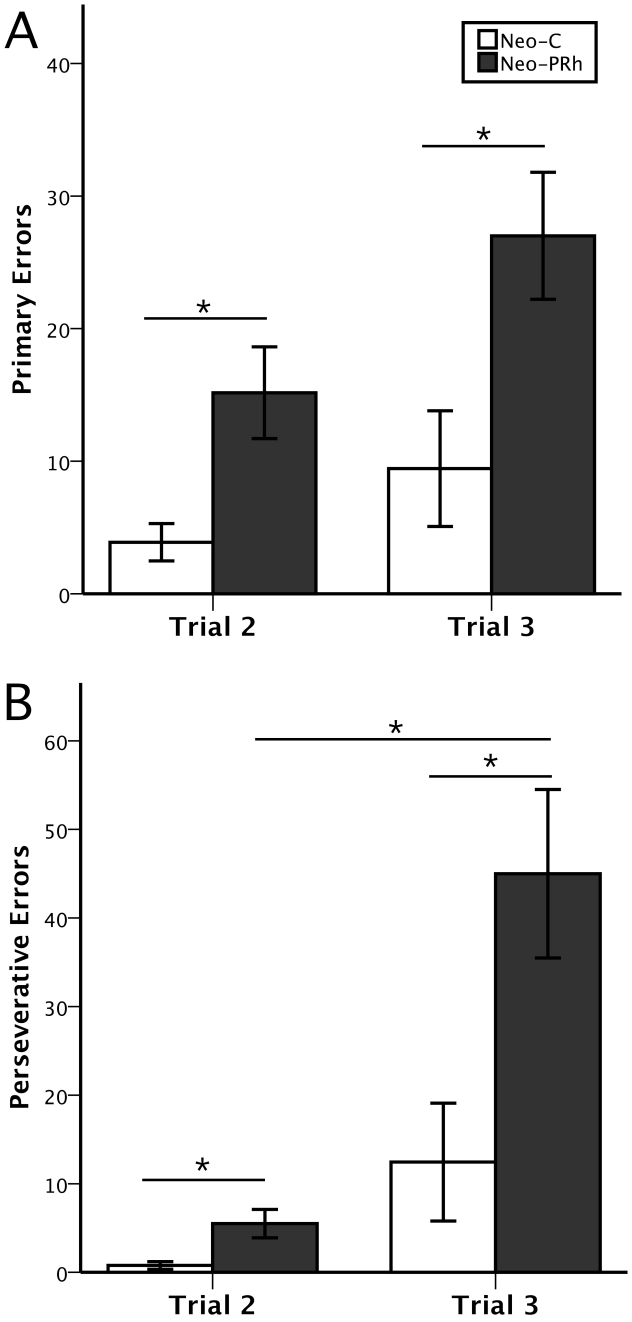


Figure 3. Object Self-Ordered Task Performance.



Manuscript in prep

Impaired cognitive flexibility after neonatal perirhinal lesions in rhesus macaques

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Abstract

Previous research indicated that monkeys with neonatal perirhinal lesions (Neo-PRh) were impaired on working memory tasks that generated proactive interference, but performed normally on working memory tasks devoid of interference (Study 2; Weiss, Nadji, & Bachevalier, 2016). This finding suggests that the early lesions disrupted cognitive processes important for resolving proactive interference such as behavioral inhibition and/or cognitive flexibility. To distinguish between these possibilities, the same Neo-PRh monkeys and their controls were trained to perform the Intradimensional/Extradimensional attentional set-shifting task (Dias et al., 1997; Roberts et al., 1988). The results indicated that Neo-PRh monkeys completed the simple and compound discrimination stages, the intradimensional shift stage, and all reversal stages comparably to controls, but made significantly more errors on the extradimensional shift stage of the task. These data indicate that impaired cognitive flexibility is the likely source of increased perseverative errors made by Neo-PRh monkeys when performing WM tasks, rather than impaired behavioral inhibition, and imply that the perirhinal cortex may play a unique and critical role in the development of attentional set shifting abilities.

Introduction

A recent study reported that adult monkeys with neonatal lesions of the perirhinal cortex (PRh) had working memory (WM) impairments that were characterized by a tendency to make perseverative errors on tasks that generated proactive interference (Weiss, Nadji, & Bachevalier, 2016). However, the same Neo-PRh animals were unimpaired when tested with a WM task that was devoid of interference (Weiss, Nadji & Bachevalier, 2016). Therefore, it is unlikely that impaired working memory processes could cause this behavioral deficit. Instead, these data suggest that the Neo-PRh lesions may have resulted in difficulty resolving proactive interference.

Resolving proactive interference requires inhibition to suppress behavioral responses based on "old" information, and flexibility to shift cognitive resources towards learning/remembering "new" information (Jha et al., 2004; Konishi et al., 2003). Therefore, one interpretation of the increase in perseverative errors made by the Neo-PRh monkeys is that the impairment could be due to a failure to suppress the influence of previously acquired stimulus-reward associations (behavioral inhibition), resulting in repetitive tendencies. A second interpretation is that the impairment could be due to difficulty shifting attention towards new stimulus-reward associations (cognitive flexibility), resulting in a tendency to choose the previously rewarded stimulus.

Lesion studies in monkeys have already demonstrated a double-dissociation between behavioral inhibition supported by the orbitofrontal cortex (OFC), and cognitive flexibility supported by the ventrolateral prefrontal cortex (vlPFC) (Bissonette, Powel, & Roesch, 2013; Burnham et al., 2010; Dias, Robbins, & Roberts, 1997; Monchi et

al, 2001; Rogers et al., 2000). Given that the PRh has robust interconnections with both of these cortical areas (Barbas & Pandya, 1989; Lavenex et al., 2002; Petrides & Pandya, 2002; Suzuki & Amaral, 1994a, 1994b), it is possible that the PRh also plays a role in mechanisms underlying behavioral inhibition and/or cognitive flexibility. Therefore, the goal of this study was to distinguish between these possible explanations by characterizing the ability of the same Neo-PRh monkeys to perform a task that taps both capacities: the Intradimensional-Extradimensional set-shifting paradigm (ID-ED) (Roberts, Robbins, & Everitt, 1988).

Methods

The Institutional Animal Care and Use Committee (IACUC) at Emory University in Atlanta, GA approved all experimental protocols. All guidelines specified in the NIH Guide for the Care and Use of Laboratory Animals (National Research Council (US), 2011) were strictly followed.

Subjects

Eleven adult rhesus macaques, aged 9-16 years, participated in this experiment (7 females, 4 males). Between 7-12 days postnatal, 6 monkeys received bilateral injections of ibotenic acid to the perirhinal cortex (group Neo-PRh; 3 females, 3 males), and 2 received sham surgeries (group Neo-C; 1 female, 1 male). One animal did not undergo any surgical or anesthetic procedures (Neo-UC; 1 female). All of these subjects received the same rearing conditions, which included extensive socialization

opportunities with age-matched peers and human caregivers (for detailed description of rearing procedures see Goursaud & Bachevalier, 2007; Raper et al., 2013). All monkeys, except Neo-C-1, were born at the Yerkes National Primate Research Center (Lawrenceville, GA). Neo-C-1 was born at the University of Texas M.D. Anderson Cancer Center Science Park (Bastrop, TX), and moved to the Yerkes NPRC.

Two additional monkeys received sham operations in adulthood (Adult-C; 1 female; 1 male) and were available to participate in behavioral testing. These Adult-C animals were mother-raised in a large colony of macaques at the Yerkes NPRC Field Station under a semi-naturalistic environment (see Raper et al., 2013 for more details), and moved to indoor pair housing between 2-4 years of age.

At the time of this experiment, all monkeys were housed individually in rooms with 12-hour light/dark cycles (7AM/7PM), fed Purina Old World Primate chow (formula 5047) and supplemented with fresh fruit enrichment. During testing, the food ration was given once daily following testing, and adjusted individually to ensure that the animals were motivated to perform on the task, and maintained their weight at 85% or above of their free-feeding weight. Water was given ad libitum.

Neuroimaging and Surgical Procedures

Between 10-12 days of age, subjects in groups Neo-PRh and Neo-C underwent surgery to create excitotoxic lesions of the perirhinal cortex using ibotenic acid, or sham operations, respectively. Animals in the Adult-C group were between 6-12 years of age at the time of their surgeries.

The brain was imaged with a 3T Siemens Magnetom Trio system (Siemens Medical Solutions, Malvern, PA at YNPRC) using a 5cm surface coil. At both time points, two sets of images were obtained: 1) high-resolution structural T1 images (3D T1-weighted fast spoiled gradient (FSPGR)-echo sequence, TE=2.6ms, TR=10.2ms, 25° flip angle, contiguous 3mm sections, 12cm FOV, 256x256 matrix; image sequences acquired in 3 series offset 1mm posterior); and 2) Fluid Attenuated Inversion Recovery images, (FLAIR, TE = 140ms, TR = 1000ms, inversion time (TI) = 2200ms, contiguous 3mm sections, 12cm FOV, 256 x 256 matrix). The T1-weighed images were used to calculate the injection sites and the FLAIR images were used to estimate the extent of PRh damage as well as damage to adjacent structures, as described in the section below.

Throughout the duration of the pre-surgical MRI scans, subjects were sedated (10mg/kg of 7:3 Ketamine Hydrochloride, 100mg/ml, and Xylazine, 20mg/ml, administered i.m.) and intubated to allow inhalation of isoflurane (1%-2%, v/v) and maintain in an appropriate plane of anesthesia. The subject's head was restrained in a stereotaxic apparatus and an IV drip (0.45% NaCl and dextrose) was used to maintain normal hydration. Vital signs (heart and respiration rates, blood pressure, body temperature and expired CO2) were constantly monitored during the scan and surgical procedures that followed.

Surgical Procedures

Following the pre-surgical scans, animals were immediately transported to the operating room and maintained throughout the surgical procedure with Isoflurane gas

(1%-2%, v/v, to effect), which were performed under deep anesthesia using aseptic conditions. The scalp was shaved and cleaned with chlorhexidine diacetate (Nolvasan, Pfizer). Bupivacaine Hydrochloride (Marcaine 25%, 1.5ml), a long-lasting local anesthetic, was injected along the planned midline incision of the scalp, which extended from the occipital to the orbital ridges. Bilateral craniotomies (1cm wide x 2.5cm long) were made above the areas to be injected. The Neo-PRh group was given injections 2mm apart along the rostral-caudal length of the perirhinal cortex using 0.4µl ibotenic acid (Biosearch Technologies, Novato, CA, 10mg/ml in PBS, pH 7.4, at a rate of 0.4µl/min).

Animals in the Neo-C and Adult-C groups underwent the same procedures, except that the injection needles were not lowered in the brain. The dura, galea, and skin were closed in anatomical layers and the animals removed from isoflurane, extubated, and closely monitored until complete recovery from anesthesia. Analgesic (acetaminophen, 10mg/kg PO) was given QID for 3 days after surgery. Additionally, animals received dexamethazone sodium phosphate (0.4mg/kg IM) to reduce edema and Cephazolin (25mg/kg IM) SID starting 12h prior to surgery and ending 7 days after to prevent infection.

Approximately one week after surgeries, animals received a second neuroimaging session using the same procedures as those they received for the pre-surgical scans.

Lesion Assessment

Histological post-mortem evaluations of the brain of the animals in this study are not yet available. Instead, lesion extent was estimated using MRI images (coronal FLAIR) acquired 1-week post-surgery. In this post-surgical scan, edema and cell death caused by the excitotoxin injections are visible as hypersignals, regions of increased signal due to cerebrospinal fluid accumulation in the injected areas. Lesion extent was evaluated with methods described in details by Zeamer et al. (2015) and briefly summarized here. After identifying the areas of hypersignals on each MR images through the perirhinal cortex, extent of hypersignals were plotted onto matching coronal sections of a normal monkey brain. The surface area (in pixels^2) of damage to the left and right perirhinal cortex and any unintended damage to adjacent structures was then measured in Image J®. Calculations of the percentage of volume damage were done by dividing the volume of damage to the perirhinal cortex by the volume of the perirhinal cortex in a normal monkey of the same age and multiply by 100. A similar procedure was used to calculate additional damage to adjacent structures. See Table 1 for a summary of extent of intended and unintended damage and Figure 1 for a representative case (Neo-PRh-6).

Behavioral Testing

Prior to participating in this study, all subjects had prior experience with cognitive tests including concurrent discrimination learning, reinforcer devaluation, object delayed reversal learning, safety signal learning, and emotional regulation (Alghrim, Raper, Johnson & Bachevalier, in prep). The Neo-PRh, Neo-C and Neo-UC monkeys had additional experience with tests of object recognition (Zeamer et al., 2015;

Weiss & Bachevalier, 2016), working memory (Weiss, Nadji & Bachevalier, 2016), perceptual discrimination and familiarity judgments (Weiss et al., in preparation; see Study 1).

Apparatus

The ID-ED task was conducted in a soundproof testing chamber with an automated testing apparatus. This apparatus consisted of a 3M Microtouch touschreen monitor and MedAssociates mini M&M dispenser controlled by a custom-written program using Presentation software. Before beginning the ID-ED task, monkeys were acclimated to the testing chamber, the touchscreen, and the sound of the reward (M&M) dispenser in 15-min daily sessions for 3 consecutive days. After these sessions, the animals readily triggered the screen and ate the rewards as they were dispensed.

Interdimensional-Extradimensional (ID/ED) Set Shifting Task

The Interdimensional-Extradimensional (ID/ED) Set Shifting task was based on the Wisconsin Card Sort paradigm and closely resembled the version in the CANTAB battery of tasks (Roberts, Robbins, & Everitt, 1988; Sahakian & Owen, 1992). For this study, a daily session consisted of 60 trials separated by 30s inter-trial intervals. Each trial required a choice between two stimuli, one associated with a food reward (+) and the other not (-). The left-right positions of the rewarded and non-rewarded stimuli were varied pseudorandomly across the 60 daily trials. Monkeys learned a series of discrimination problems schematically illustrated in Figure 2 and advanced from one

stage to the next after reaching the criterion of 10 correct trials in a row. If the monkey did not reach criterion within the daily testing session, they restarted the next day at the same stage but with their number of errors reset to zero. We recorded the number of errors to reach the learning criterion for each stage.

The first stage was a simple discrimination (SD1) between two blue shapes (S_1+ and S_2-). This stage was repeated a second time (SD2) using novel stimuli (S_3+ and S_4-) to ensure that the animals had fully acclimated to the testing chamber and were sufficiently motivated to complete 60 trials each session. SD2 was followed by a series of 3 reversals (SRs) using the same stimuli but with the reward contingency between S+ and S- switching for each reversal. Reversals rely on behavioral inhibition and require subjects learn to suppress responding towards a previously reinforced stimulus and switch responding to a previously non-reinforced stimulus. Once monkeys completed three reversals, a second dimension was introduced to the stimuli, that is the blue shapes were overlaid with orange lines (L_1 and L_2). This third stage involved discrimination between compound (shape+line) stimuli (compound discrimination, CD). Importantly, on half the trials L_1 overlay S_1 and L_2 overlay S_2, and on the other half L_1 overlay S_2 and L_2 overlay S_1. Therefore, in the CD stage monkeys learned to respond selectively to the S+ shape regardless of which line (L_1 or L_2) it is paired with. When the monkeys learned this new discrimination, they then completed another series of three reversals (compound reversals, CR). Following the CR stage was an Intradimensional Shift (IDS), in which a new set of compound shape-line stimuli were introduced, and monkeys learned to respond to a new shape (S+) and to continue ignoring the new lines.

Upon completing the IDS, there was another series of three reversals between the S+/S- (intradimensional reversals, IDR). The final stage was an Extradimensional Shift (EDS) in which a new set of compound shape-line stimuli were introduced, but now monkeys were rewarded for choosing a specific line stimulus (L+) rather than the shape. Performance on the EDS stage assesses cognitive flexibility.

Data Analysis:

The errors of Adult-C and Neo-C groups were compared on all stages of the task using independent-sample t-tests. In no instances did the group differences reach significance, and so data from these groups were combined to form a single comparison group for all subsequent analyses (group Control). Additionally, the number of errors made by the Neo-UC animal fell within the standard deviations of group Neo-C and Adult-C for all stages, and so data from this animal was also included in the Control group.

To assess group differences in the ability to learn the reversal contingencies across stages, we compared the total number of errors to complete each series of reversals using a Group x Reversal type ANOVA with repeated measures for the second factor. Planned comparisons between groups for each reversal type were run using independent-sample t-tests, and between stages for the each group individually using paired-samples t-tests.

Similarly, to assess group differences in the ability to learn the simple (SD) and compound discrimination (CD) problems as well as the intradimensional (ID) and

extradimensional (ED) discrimination problems, the numbers of errors across all discrimination problems were analyzed using a Group X Stage ANOVA with repeated measures for the second factor. Additional planned independent-sample t-tests were run between groups for each discrimination stage individually, and paired-sample t-tests between performances of the same group on different stages. Effect sizes were reported for all ANOVAs using partial eta squared (η_p^2). Effect sizes were reported for all T-tests using Cohen's *d* (d_{Cohen}).

Analyses were also run using sex as a second independent factor to determine whether there were any female/male differences among the groups. None of the analyses revealed significant sex effects, and so both sexes were combined for all analyses reported in the Results section.

Finally, bivariate Pearson correlations were run to determine if the extent of PRh damage, or unintended damage in the adjacent entorhinal cortex (ERh), was related to the number of errors to reach criterion at each stage of the ID/ED task.

Results

The numbers of errors to complete each reversal stage is illustrated in Figure 3. Analyses revealed a significant main effect of reversal stage [$F(2,18)=24.687$, $p=0.001$, $\eta_p^2=0.733$] but no significant main effect of group [$F(1,9)=0.01$, $p=0.921$, $\eta_p^2=0.001$] and no interaction [$F(2,18)=0.690$, $p=0.514$, $\eta_p^2=0.071$]. Planned independent-sample t-tests indicated that the groups did not differ significantly at any stage [SR: $t(9)=-0.483$, $p=0.640$, $d_{Cohen}=0.293$; CR: $t(9)=-0.114$, $p=0.912$, $d_{Cohen}=0.069$; IDR: $t(9)=0.953$, $p=0.366$,

d_{Cohen}=0.577]. However, planned paired-sample t-tests indicated that both groups made significantly less errors in the IDR stage than the CR stage [Neo-PRh: t(5)=4.089, p=0.009, d_{Cohen}=1.763; Control: t(4)=3.476, p=0.025, d_{Cohen}=1.133] and the SR stage [Neo-PRh: t(5)=4.950, p=0.004, d_{Cohen}=2.49; Control: t(4)=2.898, p=0.044, d_{Cohen}=1.62]. The number of errors made by group Neo-PRh also significantly differed between the SR and CR stages [t(5)=3.905, p=0.011, d_{Cohen}=0.939], but this difference did not reach significance for group Neo-C [t(4)=1.593, p=0.186, d_{Cohen}=0.597]

The numbers of errors to complete each discrimination stage is illustrated in Figure 4. Analyses revealed a significant Group X Stage interaction [F(4,36)=3.606, p=0.014, η_p^2=0.286], and a significant main effect of Stage [F(4,36)=7.385, p<0.001, η_p^2=0.451]. The main effect of Group did not reach significance [F(1,9)=2.032, p=0.188, η_p^2=0.184]. Planned independent-sample t-tests revealed that the groups differed significantly on the EDS stage [t(9)=-2.320, p=0.045, d_{Cohen}=1.405] but not for any of the other stages [SD1: t(9)=1.109, p=0.296, d_{Cohen}=0.672; SD2: t(9)=-1.287, p=0.230, d_{Cohen}=0.780; CD: t(9)=0.206, p=0.842, d_{Cohen}=0.124, IDS: t(9)=-1.430, 0.186, d_{Cohen}=0.866]. Additionally, paired sample t-tests indicated that both groups made more errors in the EDS stage than the IDS stage [Neo-PRh: t(5)=-3.833, p=0.012, d_{Cohen}=2.157; Control: t(4)=-6.028, p=0.004, d_{Cohen}=3.365], the CD stage [Neo-PRh: t(5)=-3.590, p=0.016, d_{Cohen}=1.964; Control: t(4)=-2.946, p=0.042, d_{Cohen}=1.098], and the SD2 stage [Neo-PRh: t(5)=-3.310, p=0.021, d_{Cohen}=1.372; Control: t(4)=-3.121, p=0.035, d_{Cohen}=1.37].

Correlation with lesion extent

The extent of PRh damage was not significantly correlated with the number of errors on any stage [SD1: r=-0.804, p=0.054; SD2: r=0.335; p=0.516; SR: r=0.448, p=0.373; CD: r=0.670, p=0.145; CR: r=0.759, p=0.080; IDS: r=0.249, p=0.634; IDR: r=0.756, p=0.082; EDS: r=0.697, p=0.124]. Similarly, the extent of unintended entorhinal cortex damage was not significantly correlated with the number of errors on any stage [SD1: r=-0.021, p=0.968; SD2: r=0.469; p=0.349; SR: r=0.246, p=0.639; CD: r=0.433, p=0.392; CR: r=-0.063, p=0.905; IDS: r=0.418, p=0.409; IDR: r=-0.105, p=0.843; EDS: r=0.356, p=0.489]. However, it must be acknowledged that the lesions in the 6 Neo-PRh monkeys were similar in extent and had limited variability, ranging only between 70%-85% (see Table 1). This lack of variability limits our ability to interpret the correlations between lesion extent and task performance.

Discussion

This is the first study to date that has investigated the impact of neonatal PRh lesions on cognitive and behavioral inhibition using the ID/ED set shifting ask. The results indicated that Neo-PRh lesions had little impact on the ability of adult monkeys to acquire novel visual discriminations in the SD, CD, and IDS stages, or to complete the reversal stages. By contrast, they significantly impaired the EDS stage. The results indicate that mechanisms important for visual discrimination learning and behavioral inhibition remained in the normal range, but mechanisms mediating cognitive flexibility

were significantly impacted by the early PRh lesions. These findings are discussed in turn.

Visual Discrimination Learning

Visual discrimination learning involves the formation of stimulus-response associations. In the SD, CD, and IDS stages, monkeys learned which of two stimuli to respond to in order to obtain a reward, and which to avoid. Monkeys with Neo-PRh lesions completed the visual discrimination stages of the ID/ED task as quickly and accurately as controls. These data confirmed earlier findings from the same animals when tested on the 60-pair concurrent discrimination task, and indicate that Neo-PRh lesions do not impair, but actually facilitate, simple discrimination learning (unpublished data). Monkeys with PRh lesions created in adulthood are also able to perform similar discrimination tasks normally (Hampton & Murray, 2002; Thornton, Rothblat, & Murray, 1997;Gaffan & Murray, 1992). Taken together, these data indicate that the PRh does not play a significant role in stimulus-response association learning.

Behavioral Inhibition

Reversal learning involves inhibition of previously acquired stimulus-reward associations. In the Reversal stages, monkeys learned to switch their response strategies, that is avoid the stimulus previously rewarded and select the previously unrewarded stimulus. In the current study, monkeys with Neo-PRh lesions were unimpaired on all reversal stages of the ID/ED task. This finding corroborates data from

an earlier study with the same Neo-PRh animals in which they were unimpaired in learning 5 concurrent object discrimination reversal problems (unpublished data), and contrasts with the impaired performance of monkeys with adult-onset PRh lesions on similar reversal tasks (Hampton & Murray, 2002 ; Murray, Baxter, & Gaffan, 1998). However, the different outcomes following neonatal and adult-onset PRh lesions could be related to two important procedural differences between the studies. First, the lesions in the adult monkeys were created by surgical aspiration, whereas the lesions in the infant monkeys were created by injection of neurotoxin. An important difference between these two methods is that the neurotoxin injection destroys only the neurons it contacts, whereas the surgical aspiration also destroys fibers that pass through the area. Studies directly comparing the impact of these lesion techniques in other MTL areas indicated that more severe deficits followed aspiration lesions than neurotoxic lesions (Glenn et al., 2005; Meunier et al., 1999). Second, the adult PRh lesions encompassed the entire PRh and large portions of the entorhinal cortex, whereas the neonatal PRh lesions did not. Therefore, damage to other MTL areas, rather than the PRh, could also be the cause of the reversal learning impairments in monkeys with adult-onset PRh lesions (see Zola, Squire & Ramus, 1994). Although data from the current study suggest that the PRh does not play a role in behavioral inhibition, comparisons with the adult data highlight a need for future studies to clarify the role of the PRh in reversal learning and behavioral inhibition when lesions are made in adulthood.

Cognitive Flexibility

Cognitive flexibility involves the ability to switch attention to different sources of information, especially when behavioral responses become unsatisfactory or inadequate. The EDS stage requires flexibility to ignore the previously rewarded dimension of the stimuli (shape) and shift attention to the previously ignored dimension of the stimuli (line). Neo-PRh monkeys had significant difficulty shifting their response strategies during the EDS stage, as indicated by their high error rates. Compared with the normal performance on reversal learning (behavioral inhibition) reported above, these data suggest that Neo-PRh lesions impaired mechanisms of cognitive flexibility.

Relationship to perseverative responses

Performance of the Neo-PRh monkeys on WM tasks that generated proactive interference was characterized by greater tendencies for perseverative errors, yet the same animals were unimpaired on a WM task that was devoid of interference (Study 2, Weiss, Nadji & Bachevalier, 2016). This finding indicated that the early lesions did not impact WM processes per se but rather altered executive cognitive processes other than WM. The current study provided a potential explanation for the increase in perseverative errors in Study 2. Neo-PRh monkeys had significant difficulty learning to shift their attention to new perceptual features in the EDS stage, but were able to complete visual discrimination and reversal stages as quickly and accurately as controls. These findings imply that deficient cognitive flexibility is a likely source of the perseverative errors in WM task with high proactive interference.

The critical involvement of the dorsolateral PFC is well established in WM processes of monitoring/manipulation (Alexander & Goldman, 1978; Goldman & Rosvold, 1970; Miller & Cohen, 2003; Petrides & Milner, 1982; Petrides, 1991a, 1991b, 1995, 2000), whereas processes involved with perseveration and cognitive flexibility are reliant on ventrolateral and medial PFC regions (Bissonette et al., 2013; Burnham et al., 2010; Monchi et al., 2001; Rogers et al., 2000). It is therefore noteworthy that the PFC regions critical for cognitive flexibility receive comparably heavier perirhinal inputs than do the regions involved in WM (dorsolateral PFC), and comparably fewer from other areas like inferotemporal cortex, parahippocampal cortex, and the hippocampus (Cavada et al., 2000; Croxson et al., 2005; Kondo et al., 2005; Lavenex et al., 2002; Saunders et al., 2005; Suzuki & Amaral, 1994a, 1994b). This distinct pattern of PRh-PFC anatomical connectivity could explain why removal of the PRh had a more profound impact on mechanisms relying on the ventrolateral PFC (cognitive flexibility) than mechanisms relying on dorsolateral PFC (working memory).

Conclusion

Infancy represents a stage of development characterized by increased levels of neural plasticity (for reviews see Kolb & Gibb, 2007; Takesian & Hensch, 2013). Perturbation of the brain at this early stage of development may lead to increased opportunity for compensation, but may also increase vulnerability to maldevelopment. In the current study, Neo-PRh lesions profoundly impaired set shifting, whereas data on the effects of extended damage to MTL structures (including the PRh) in adulthood

indicates that set-shifting abilities are spared (Owen et al., 1991). This dissociation suggests that mechanisms of cognitive flexibility were more affected by the early damage than after the adult-onset damage. Given the early timing of the neonatal lesions, the deficits in cognitive flexibility may instead represent downstream effects of the neonatal lesions on the normal development and maturation of the brain areas important for flexible cognition and preventing perseverative responding, the vlPFC (Baxter et al., 2009; Bissonette et al., 2013; Burnham et al., 2010; Dias et al., 1997; Iversen & Mishkin, 1970; Monchi et al., 2001; Owen et al., 1991; Rogers et al., 2000). The protracted anatomical and functional development of this area has been well established (Conklin et al., 2007; Fuster, 2002; Kolb & Gibb, 2011), and a number of morphological and neurochemical changes in the lateral PFC have been reported following early damage to other MTL structures (Bertolino et al., 1997; Chlan-Fourney, Webster, Felleman, & Bachevalier, 2000; Meng et al., 2013; Tseng et al., 2008). The current findings indicate that early PRh damage has a profound impact on the development of flexible cognition and suggest altered functionality of the vlPFC. Future studies will need to assess the effects of Neo-PRh lesions on prefrontal morphology, and to document whether there are windows of increased vulnerability during which early lesions have differential impacts on the development of the PFC.

Figure Captions:

Figure 1. MR images shown at three rostro-caudal levels through the perirhinal cortex are pre-surgical structural T1 weighted coronal images (left column) and 1 week post-surgical coronal FLAIR images (right column) for a representative case. Visible in the post-surgical images are regions of hypersignal (white areas) that are indicative of edema and cell damage resulting from the ibotenic acid injection. Arrows point to the rhinal sulcus (left column) and to areas of hypersignal (right column). See Zeamer et al (2015), Weiss & Bachevalier (2016), Weiss, Nadji & Bachevalier (2016), Alhgrim, Raper, Johnson, & Bachevalier (in prep), and Weiss, Guo, Richardson & Bachevalier (in prep) for additional surgical cases.

Figure 2. In the ID-ED paradigm, monkeys learned the series of discrimination problems shown here. As they progressed through the stages, reinforcement contingencies were switched, requiring subjects to spontaneously shift their response strategies. Importantly, some rule shifts measured behavioral inhibition by requiring monkeys to reverse S+/S- relationships between two stimuli (SR, CR, and IDR stages) whereas other rule shifts measured cognitive flexibility by requiring monkeys to reorient their responses towards a feature of a stimulus that was previously ignored/irrelevant (EDS stage).

Figure 3. The number of errors for the Neo-PRh group (shaded bars) and the Control group (open bars) to complete the Simple Reversal (SR), Compound Reversal (CR), and

Intradimensional Reversal (IDR) Stages. Bars represent +/- 1 SE.

Figure 4. The number of errors for the Neo-PRh group (shaded bars) and the Control group (open bars), on the Simple Discrimination (SD), Compound Discrimination (CD), Intradimensional Shift (IDS), and Extradimensional Shift (EDS) Stages of the ID-ED task. Bars represent +/- 1 SE, and *indicates significant group differences ($p<0.05$)

Figure 1: Pre- and Post-Surgical MR Images from a representative case (Neo-PRh-6).

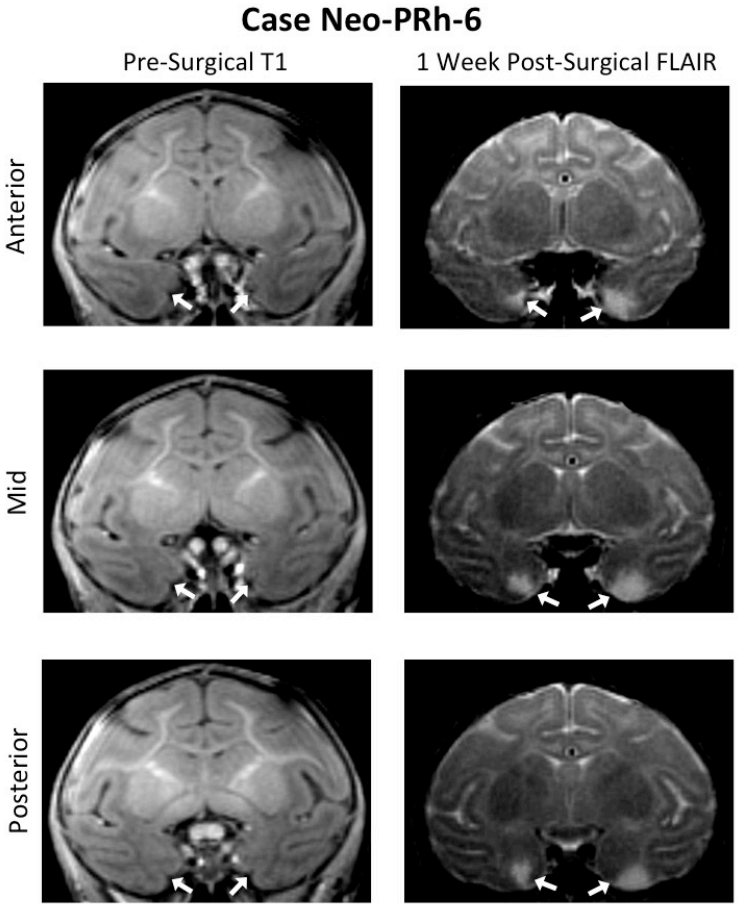

Figure 2: Intradimensional-Extradimensional (ID/ED) Set Shifting Task Schematic**.**

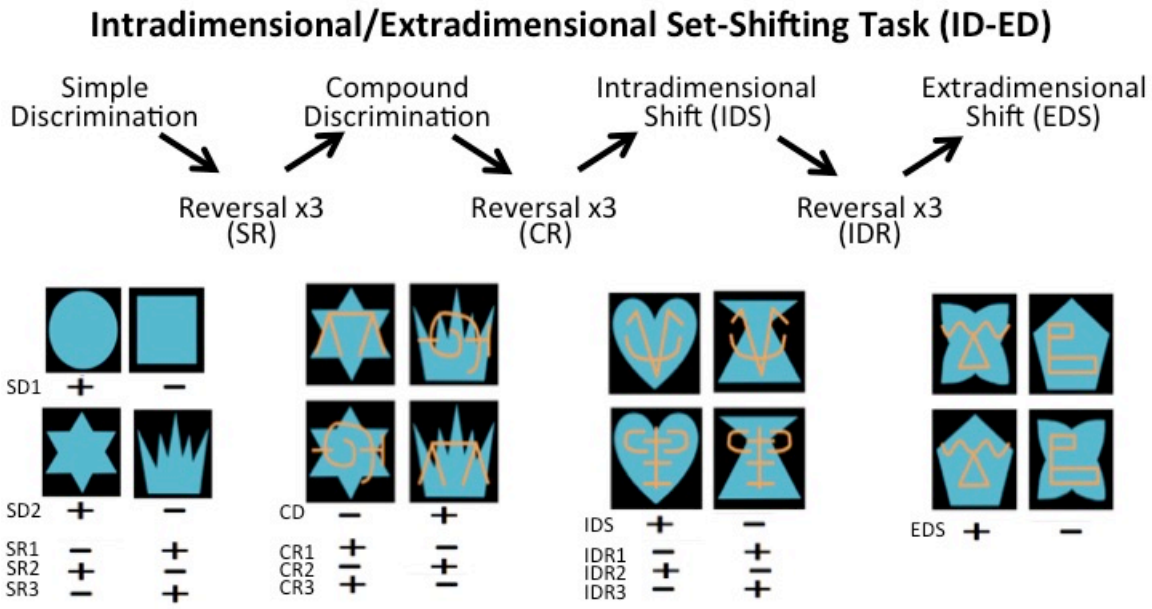

Figure 3: Reversal Stages

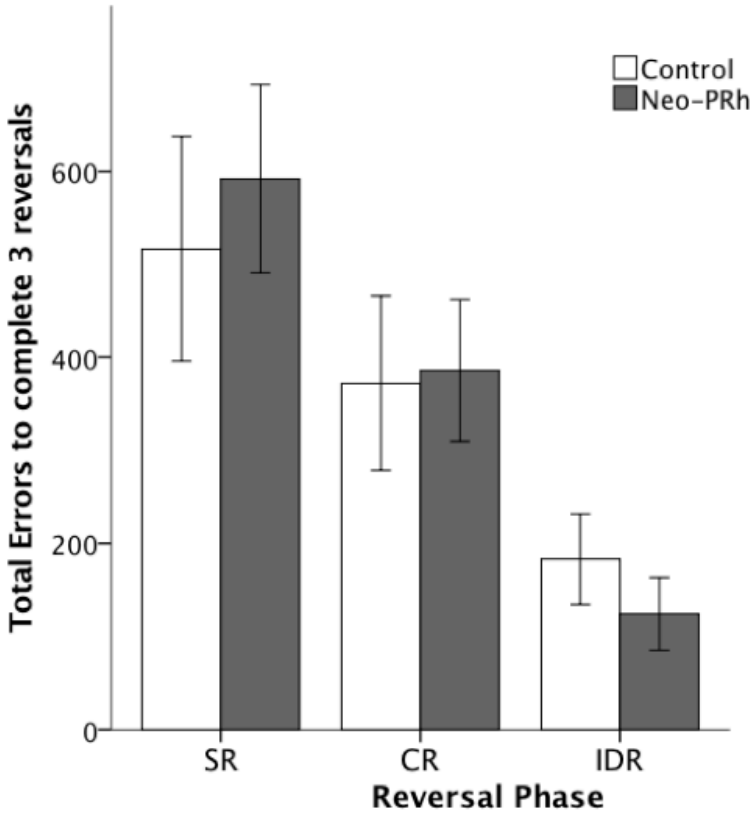

Figure 4: Discrimination Stages.

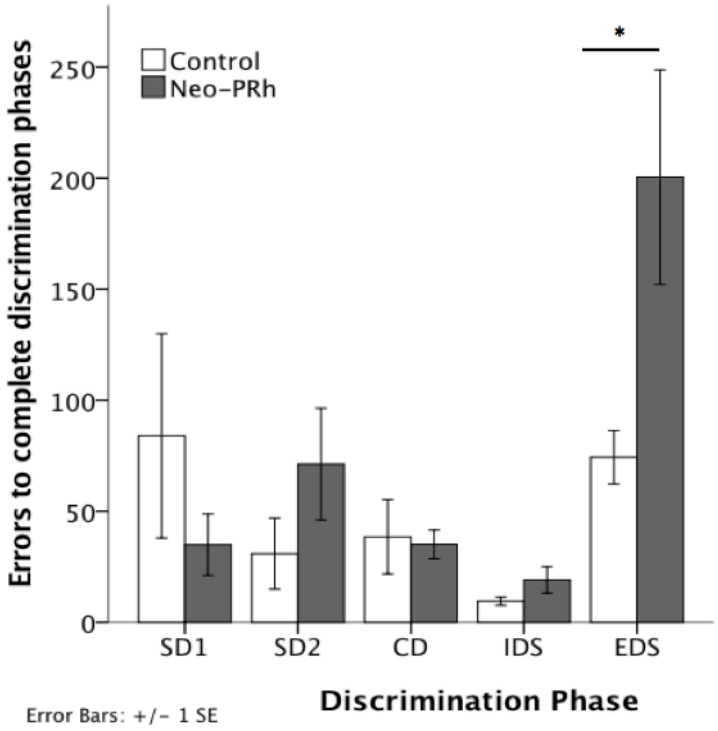

Table 1: Summary of Lesion Extent.

Subjects	PRh				ERh				TE			
	L%	R%	X%	W%	L%	R%	X%	W%	L%	R%	X%	W%
Neo-PRh-1	89.76	79.91	83.34	69.04	28.51	2.28	15.39	0.65	4.53	9.70	7.11	0.44
Neo-PRh-2	68.16	70.58	69.37	48.11	17.72	20.65	19.19	3.36	0.14	0.06	0.10	0.00
Neo-PRh-3	65.45	81.02	73.23	53.02	7.72	3.12	5.42	0.24	0.26	3.39	1.82	0.01
Neo-PRh-4	59.40	74.73	67.06	44.39	11.55	17.84	14.69	2.06	0.72	2.62	1.67	0.02
Neo-PRh-5	75.90	66.81	71.35	50.71	38.60	29.86	34.32	11.53	0.72	0.41	0.57	0.00
Neo-PRh-6	74.12	80.31	77.22	59.53	25.34	43.64	34.49	11.06	0.37	2.93	1.65	0.01
Average	*72.13*	*75.06*	*73.60*	*54.13*	*21.57*	*19.57*	*20.57*	*4.87*	*1.12*	*3.19*	*2.15*	*0.08*

Subjects	TH/TF				AMY				HF			
	L%	R%	X%	W%	L%	R%	X%	W%	L%	R%	X%	W%
Neo-PRh-1	0.00	0.00	0.00	0.00	8.24	10.86	9.55	0.89	0.13	2.39	1.26	0.00
Neo-PRh-2	0.00	0.00	0.00	0.00	0.00	2.76	1.38	0.00	0.00	0.00	0.00	0.00
Neo-PRh-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.27	0.14	0.00
Neo-PRh-4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Neo-PRh-5	7.02	3.93	5.47	0.28	0.00	0.00	0.00	0.00	3.37	0.00	1.68	0.00
Neo-PRh-6	0.00	0.00	0.00	0.00	3.78	4.17	3.97	0.16	3.32	0.32	1.77	0.01
Average	*1.17*	*0.66*	*0.91*	*0.05*	*2.00*	*2.96*	*2.48*	*0.18*	*1.12*	*0.50*	*0.81*	*0.00*

Scores are estimates of intended and unintended damage following Neo-PRh lesions for each case. L% = percent damage to left hemisphere; R% = percent damage to right hemisphere; X% = average damage to both hemispheres; W% = weighted damage to both hemispheres (W% = (L% X R%)/100). PRh, perirhinal cortex; ERh, entorhinal cortex, TE, temporal cortical area; TH/TF, parahippocampal cortex; AMY, amygdala; HF, hippocampal formation. Lesion extents from cases Neo-PRh-1 thru Neo-PRh-6 were previously reported by Zeamer et al. (2015).

General Discussion

The three manuscripts presented in this report represent a step forward in our understanding of the cognitive processes that are supported by the perirhinal cortex. Earlier reports already indicated that the PRh is critical to support the development of recognition memory (Weiss & Bachevalier, 2016; Zeamer et al., 2015), and performance on working memory tasks that generated proactive interference (Weiss, unpublished masters thesis). The central goal of this dissertation project was to advance our interpretation of these impairments by clarifying the long-term impact of neonatal PRh lesions on visual perception and familiarity judgments (Study 1), on a working memory task devoid of proactive interference (Study 2), and on cognitive flexibility/behavioral inhibition (Study 3). Study 1 indicated that neonatal PRh lesions had a significant impact on the development of familiarity mechanisms, but spared visual perception. Study 2 demonstrated that the same Neo-PRh lesions did not alter working memory processes per se, but rather increased the tendency to make perseverative errors. Study 3 provided evidence that impaired cognitive flexibility was a likely source of the increased perseverative errors made by Neo-PRh monkeys when performing WM tasks with proactive interference. Taken together, the results of this project shed light on neural mechanisms that support normal cognitive development.

The next sections will summarize what can be concluded on the role of the PRh in the normal development of mechanisms that support recognition memory, working memory and proactive interference. The following section will distinguish the role of the PRh from the role of the hippocampus in the development of each of these cognitive

domains. The last section will discuss how nonhuman primate models can inform our understanding of the neural bases of human neuropsychiatric disorders.

The role of the PRh in recognition memory

Early influential work with nonhuman primate lesion models inspired by clinical observations of patients with anterograde amnesia (Scoville & Milner, 1957), initially implicated the hippocampus as a key structure in support of recognition memory (Iverson, 1976; Mishkin, 1978). However, data from a series of studies following these hallmark reports subsequently identified the PRh as another MTL structure that critically contributes to recognition memory processes. The key observation emerging from these studies was that adult monkeys with PRh lesions had recognition memory impairment almost as severe as that following damage that encompassed large areas of the MTL (Eacott et al., 1994; Meunier et al., 1993; Suzuki et al., 1993; Zola-Morgan et al., 1989; Zola-Morgan & Squire, 1985), and more severe than lesions restricted to the hippocampus (Alvarez, Zola-Morgan, & Squire, 1995; Murray & Mishkin, 1998; Nemanic, Alvarado, & Bachevalier, 2004). The implication was that PRh damage alone could therefore account for the memory impairments seen in many previous studies in which the PRh was included in the MTL lesions. Additional work has expanded these findings, showing that the PRh is also critical for recognition of tactile stimuli (Buffalo et al., 1999), indicating that this cortical area processes information in service of recognition from other sensory modalities, but not spatial information (Alvarado & Bachevalier, 2005; Bachevalier & Nemanic, 2008). Altogether, the studies highlighted

the unique and specific contribution of the PRh to mechanisms of recognition memory.

Furthermore, developmental studies have shown that object recognition memory emerges very early in development and is supported, at least in part, by the PRh. Infant humans and infant monkeys are able to perform memory tasks that depend on familiarity judgments as young as 1 month of age (Bachevalier, 1990; Diamond & Goldman-Rakic, 1989; Diamond, 1990). This early developing ability is impaired by neonatal PRh lesions as early as 1.5 months of age in monkeys (Zeamer et al., 2015) and worsens with maturation of the animals to persist in adulthood. Interestingly, the magnitude of the recognition memory deficit after the neonatal PRh lesions was less than following the adult-onset PRh lesions (Weiss & Bachevalier, 2016; Zeamer et al., 2015), suggesting significant functional compensation that likely occurred following the early PRh lesions. The results suggest that other brain structures may compensate for the loss of familiarity processes in the event of an early PRh malfunction. There is ample evidence of increased recovery of sensorimotor and visual function following early injury (for review see Cioni, D'Acunto, & Guzzetta, 2011), but there have been comparably fewer studies that extended this finding to cognitive systems (Goldman et al., 1970; Kolb & Gibb, 2011; Kolb et al., 2013; Miller et al., 1973). In this way, the work presented in this dissertation has enhanced our understanding of the development of recognition memory and of early brain plasticity within this system. Future studies are needed to determine the timing of critical periods during development with increased potential for functional recovery, and the factors that influence those trajectories.

The role of the PRh in WM and proactive interference

Historically, WM and 'executive functions', such as behavioral inhibition and cognitive flexibility have been regarded as the exclusive domain of the PFC (for review see Jeneson & Squire, 2012), yet new evidence suggests otherwise. Recent functional imaging studies in adult humans and monkeys reported that several MTL structures, including the PRh and hippocampus, are also activated during many WM tasks (Davachi & Goldman-Rakic, 2001; Stern, Sherman, Kirchhoff, & Hasselmo, 2001; Libby, Ekstrom, Ragland, & Ranganath, 2012; Ranganath, Cohen, Dam, & D'Esposito, 2004). Electrophysiological recording studies in monkeys have corroborated the neuroimaging findings and PRh cells are highly activated during WM tasks requiring the temporary maintenance of object representations (Lehky & Tanaka, 2007). These findings suggest that the PRh could also be important for supporting representations of familiar objects in service of WM and other executive functions. The work presented in this dissertation has significantly enhanced this picture by highlighting the unique role of the PRh in the development of prefrontal functions.

The evidence reported in Study 2 and Study 3 indicated that the PRh is more important for the development of mechanisms that help resolve proactive interference and/or control perseverative responding, than for mechanisms supporting object representations in working memory. It is possible that other brain areas, such as the hippocampus or cortical area TE/TEO, could support object representations in WM in the absence of a functional PRh. Thus, although neuroimaging and electrophysiological data clearly indicate that in adult subjects the PRh is involved in WM processes, the

current data suggest that, in the absence of a functional PRh in infancy, these cognitive processes appear to rely on other brain areas. In contrast, the PRh appears to play a more critical role in the development and maintenance of cognitive flexibility. This capacity is known to rely more on ventrolateral and medial prefrontal areas than dorsolateral prefrontal areas important for WM (Bissonette, Powell, & Roesch, 2013; Burnham, Bannerman, Dawson, & Southam, 2010; Monchi, Petrides, Petre, Worsley, & Dagher, 2001; Rogers, Andrews, Grasby, Brooks, & Robbins, 2000). It is also noteworthy that these ventrolateral/medial PFC regions receive comparably heavier PRh inputs than dorsolateral regions do, and comparably fewer from other areas like TE/TEO and the hippocampus (Cavada et al., 2000; Croxson et al., 2005; Kondo et al., 2005; Lavenex et al., 2002; Saunders et al., 2005; Suzuki & Amaral, 1994a, 1994b). This pattern of PRh-PFC anatomical connectivity could explain why the early removal of the PRh had a more profound impact on the development of cognitive flexibility than working memory. It is also important to note the deficit in cognitive flexibility following Neo-PRh lesions contrasts with the minimal effects on cognitive flexibility reported after widespread MTL damage in adulthood, including the PRh (Owen, Roberts, Polkey, Sahakian, & Robbins, 1991). Thus, taken together the data suggest that the absence of functional PRh inputs to the ventrolateral and medial PFC early in life could specifically alter the maturation of the ventrolateral/medial prefrontal cortical areas leading to the maldevelopment of this cognitive ability, while sparing WM ability mediated by the dorsolateral PFC.

As a whole, this body of work provides clear evidence of the detrimental long-term impacts of early perirhinal damage on some prefrontal functions. To advance these

proposals, it will be necessary for future studies to determine the extent to which there may be periods during development of increased vulnerability during which the effects of damage are more widespread, and the factors that influence those trajectories.

Comparisons with the effects of neonatal hippocampal lesions

A large body of work has linked the hippocampus with mechanisms of recognition (Malkova et al., 2000; Meunier et al., 1993, 1996; Mumby & Pinel, 1994; Nemanic et al., 2004; Wan et al., 1999), working memory (Libby et al., 2012; Petrides, 2000; Ranganath et al., 2004;), and interference (Butterly et al., 2012; Shapiro and Olton, 1994). Given the strong anatomical connections between the PRh and the hippocampus (Insausti et al., 1987; Suzuki & Amaral, 1994b; Suzuki, 1996), a possible interpretation of the current findings could be that the Neo-PRh lesions disconnected the hippocampus from receiving its flow of information and yielded the impairments observed in the Neo-PRh monkeys. Therefore, comparisons between the effects of neonatal PRh and neonatal hippocampal lesions are necessary to distinguish the roles of these two structures in the development of recognition, working memory, and interference. Insights from these comparisons are reviewed below, and summarized in Table 1.

Recognition

Earlier studies on the development of recognition memory in both humans and monkeys have indicated that recognition memory is present very soon after birth, as

demonstrated by the early emerging capacity to recognize familiar objects in both species (Bachevalier, Brickson, & Hagger, 1993; Gunderson & Sackett, 1984; Pascalis & de Schonen, 1994; Rose, Feldman, & Jankowski, 2009). In addition, and evidence of the profound, immediate, and long-lasting impact of neonatal PRh lesions on recognition memory (Weiss & Bachevalier, 2016; Zeamer et al., 2015). However, the presence of adult-like abilities in early infancy does not necessarily indicate that these early developing skills are mediated by the same neuronal networks that support the same function in adulthood. Supporting this proposal are data comparing the effects of neonatal hippocampal lesions and neonatal perirhinal lesions in monkeys. Neonatal hippocampal lesions produce recognition memory impairment with a delayed-onset that occurs later in development, around 18 months of age (Zeamer & Bachevalier, 2013; Zeamer et al., 2010). Thus, although this ability is mediated by the hippocampus in adulthood (Nemanic et al., 2004; Zeamer et al., 2011), the normal recognition abilities observed prior to 18 months of age suggest that brain structures other than the hippocampus may support this function in the absence of a fully functional hippocampus (Jabès, Lavenex, Amaral, & Lavenex, 2010, 2011; Payne, Machado, Bliwise, & Bachevalier, 2010). Data from the effects of neonatal PRh lesions provide support to this proposal. Thus, as compared to the neonatal hippocampal lesions, the neonatal PRh lesions yielded recognition impairment in the first month of life, and this impairment became more severe with age and persisted in adulthood. Together these data demonstrate that early in infancy recognition memory is mediated by early-developing MTL cortical areas, such as the PRh cortex, and that with further maturation,

this function becomes also associated to the progressive functional maturation of the hippocampus. Furthermore, given the evidence of the importance of the PRh for familiarity, and of the hippocampus for recollection, the developmental data suggest that familiarity mechanisms mediated by the perirhinal cortex could support the early emergence of recognition memory and dominate recognition memory processes during infancy until recollection mechanisms mediated by the hippocampus become fully mature later on and allow for stronger memory abilities. Future studies are needed to determine the factors that influence how, and under what circumstances, these two mechanisms are engaged in support of recognition, and how this interaction may be impacted by age/development.

WM and proactive interference

Although neuroimaging and electrophysiological studies have pointed to the PRh as a structure that is active during certain WM processes, evidence from Study 2 indicated that these processes do not critically depend on PRh integrity to develop normally. Rather, the data indicate that the PRh is critical to support cognitive flexibility that helps reduce the influence of proactive interference. By contrast, the role of the hippocampus appears to be more important to support WM processes (Heuer & Bachevalier, 2011; 2013) than cognitive flexibility. This dissociation in the different contribution of the PRh and hippocampus in these cognitive abilities may be related to the interactions of the PRh and hippocampus with different areas of the prefrontal cortex. Specifically, the ventrolateral/medial areas of the PFC interact with the PRh, as

compared to the dorsolateral areas of the PFC that interact more strongly with the hippocampus (Cavada et al., 2000; Croxson et al., 2005; Kondo et al., 2005; Lavenex et al., 2002; Saunders et al., 2005; Suzuki & Amaral, 1994a, 1994b). Accompanying the cognitive impairment following neonatal hippocampal lesions are morphological and neurochemical changes in the dorsolateral PFC that are not seen with adult monkeys that had received their lesions in adulthood (Bertolino et al., 1997; Chlan-Fourney, Webster, Felleman, & Bachevalier, 2000; Chlan-Fourney, Webster, Jung, & Bachevalier, 2003; Meng et al., 2013). These data imply that the cause of the WM impairment after neonatal hippocampal lesions (Heuer & Bachevalier, 2011; 2013) may related to dorsolateral PFC dysfunction, and highlight the far-reaching effects of early MTL damage on the functionality of the PFC. However, no studies to date have examined whether such anatomical changes are also seen in the ventrolateral PFC following neonatal perirhinal lesions, and so future efforts should be directed towards characterizing the morphological and neurochemical outcomes of early PRh lesions on the prefrontal cortex.

Summary

The contrasts between the effects of neonatal PRh and neonatal hippocampal lesions highlight the distinct profiles of recognition and working memory impairment that follow these two types of early lesions. In comparison to neonatal hippocampal lesions, Neo-PRh lesions yielded earlier-emerging recognition memory impairments, spared WM processes, but increased perseverative responding. Given the different

outcomes of the two lesions, we can conclude that the effects observed with Neo-PRh lesions are not simply due to a disconnection of the inputs from PRh to the hippocampus. Instead, these comparisons differentiate the role of the PRh from the hippocampus, and highlight its unique and specific role in the development of memory and cognition.

Conclusion

Current research in the areas of cell biology, genetics, and neuroscience is progressing quickly, due in part to the implementation of animal models such as the ones reviewed in this chapter. To capitalize on this new scientific knowledge, the National Institute of Mental Health implemented the Research Domain Criteria Project (RDoC), which establishes a framework to guide research projects linking brain-behavior relationships with clinical phenomena (NIMH, 2011). This change emerged because discoveries from many recent experiments have had only limited clinical impact. The source of this discrepancy is that animal models do not map clearly onto the current diagnostic categories for mental illness established by the DSM and ICD (American Psychiatric Association, 2013; World Health Organization, 1992), particularly because animal models do not replicate the full profile of clinical symptoms described by modern diagnostic tools. Instead, animal models manipulate specific neurophysiological, endocrine, biochemical, or cognitive components of psychiatric diagnostic categories, and investigate the impact of these manipulations on specific domains of human behavior and cognition, often within the context of development and environmental

influence. This theoretical framework has significant clinical relevance because specific domains of behavioral and cognitive dysfunction are common in a number of mental disorders. For example, perirhinal cortex function may be altered in Attention Deficit Hyperactivity Disorder, Autism, Dementia, Major Depressive Disorder, Schizophrenia, and Temporal Lobe Epilepsy (for reviews see Biagini et al., 2013; Godsil, Kiss, Spedding, & Jay, 2013; Machado & Bachevalier, 2003), although the timing and characteristics of behavioral and cognitive dysfunction may differ. Therefore, in accordance with this new RDoC framework, animal models that provide knowledge on the functioning of the perirhinal cortex and other MTL structures across the lifespan will also provide a better understanding of the source of the neural impact in these neuropsychiatric disorders more generally, and facilitate the design of new treatment strategies. The work presented in this dissertation is a first step in defining the role of the PRh in the development of cognitive functions. Further studies are likely to yield important discoveries identifying the key pathways involved in developmental neuropsychiatric disorders, and provide a foundation to develop more effective interventions and treatments.

Table 1. Summary of comparisons between Neo-PRh and Neo-H**.**

		Neo-PRh	Neo-H
Object Recognition			
Passive	VPC	Impairment emerges early (1.5 months), persists in adulthood[1]	Impairment emerges with delayed onset (18months)[2]
Active	DNMS	Impairment with delays longer than 10s[3]	No impairment[4]
Working Memory			
Maintenance	SU-DNMS	Transient impairment[5]	No impairment[6]
Monitoring/Manipulation	OBJ-SO	Severe impairment[5]	Severe impairment[6]
	SOMT	No impairment[5]	Severe impairment[7]
Perseverative responding?		Yes[5]	No[6]
Proactive Interference			
Behavioral Inhibition	Reversals	No impairment[8]	No impairment[9]
Cognitive Flexibility	Set-Shifting	Severe impairment[8]	Not tested

Table References: [1] Zeamer et al., 2015; [2] Zeamer et al., 2010; [3] Weiss & Bachevalier, 2016, [4] Heuer & Bachevalier, 2011; [5] Weiss, Nadji & Bachevalier, 2016 (Study 2); [6] Heuer & Bachevalier, 2011; [7] Heuer & Bachevalier, 2013; [8] Weiss, White & Bachevalier, in prep (Study 3), [9] Kazama & Bachevalier, personal communication.

References

Aggleton, J. (2012). Multiple anatomical systems embedded within the primate medial temporal lobe: implications for hippocampal function. *Neuroscience and Biobehavioral Reviews*, *36*(7), 1579–96.

Aggleton, J., Hunt, P., & Rawlins, J. (1986). The effects of hippocampal lesions upon spatial and non-spatial tests of working memory. *Behavioural Brain Research*, *19*(2), 133146.

Albasser, M. M., Poirier, G. L., & Aggleton, J. P. (2010). Qualitatively different modes of perirhinal hippocampal engagement when rats explore novel vs. familiar objects as revealed by c Fos imaging. *European Journal of Neuroscience*, *31*(1), 134–147.

Alexander, G., & Goldman, P. (1978). Functional development of the dorsolateral prefrontal cortex: An analysis utilizing reversible cryogenic depression. *Brain Research*, *143*(2), 233-249.

Alvarado, M. C., & Bachevalier, J. (2005). Comparison of the effects of damage to the perirhinal and parahippocampal cortex on transverse patterning and location memory in rhesus macaques. *The Journal of Neuroscience*, *25*(6), 1599–609.

Alvarez, P., Zola-Morgan, S., & Squire, L. R. (1995). Damage limited to the hippocampal region produces long-lasting memory impairment in monkeys. *Journal of Neuroscience*, *15*(5), 3796-3807.

Bachevalier, J. (1990). Ontogenetic Development of Habit and Memory Formation in Primates. *Annals of the New York Academy of Sciences*, *608*(1), 457–484.

Bachevalier, J., & Nemanic, S. (2008). Memory for spatial location and object-place associations are differently processed by the hippocampal formation, parahippocampal areas TH/TF and perirhinal cortex. *Hippocampus*, *18*(1), 64–80.

Bachevalier, J., Wright, A., & Katz, J. (2013). Serial position functions following selective hippocampal lesions in monkeys: Effects of delays and interference. *Behavioural Processes*, *93*, 155-166.

Baizer, J. S., Ungerleider, L. G., & Desimone, R. (1991). Organization of visual inputs to the inferior temporal and posterior parietal cortex in macaques. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *11*(1), 168–90.

Bakst, I., Morrison, J. H., & Amaral, D. G. (1985). The distribution of somatostatin-like immunoreactivity in the monkey hippocampal formation. *Journal of Comparative Neurology*, *236*(4), 423-442.

Barbas, H., & Pandya, D. (1989). Architecture and intrinsic connections of the prefrontal cortex in the rhesus monkey. *Journal of Comparative Neurology*, *286*(3), 353–375.

Barense, M., Ngo, J., Hung, L., & Peterson, M. (2012). Interactions of Memory and Perception in Amnesia: The Figure–Ground Perspective. *Cerebral Cortex*, *22*(11), 2680–2691.

Baxter, M. G., Gaffan, D., Kyriazis, D. A., & Mitchell, A. S. (2009). Ventrolateral prefrontal cortex is required for performance of a strategy implementation task but not reinforcer devaluation effects in rhesus monkeys. *European Journal of Neuroscience*, *29*(10), 2049–2059.

Berger, B., & Alvarez, C. (1994). Neurochemical development of the hippocampal region in the fetal rhesus monkey. II. Immunocytochemistry of peptides, calcium-binding proteins, DARPP-32, and monoamine innervation in the entorhinal cortex by the end of gestation. *Hippocampus*, *4*(1), 85–114.

Bertolino, A., Saunders, R., Mattay, V., Bachevalier, J., Frank, J., & Weinberger, D. (1997). Altered development of prefrontal neurons in rhesus monkeys with neonatal mesial temporo-limbic lesions: a proton magnetic resonance spectroscopic imaging study. *Cerebral Cortex*, *7*(8), 740–748.

Biagini, G., D'Antuono, M., Benini, R., de Guzman, P., Longo, D., & Avoli, M. (2013). Perirhinal cortex

and temporal lobe epilepsy. *Frontiers in Cellular Neuroscience*, *7*.

Bissonette, G., Powell, E., & Roesch, M. (2013). Neural structures underlying set-shifting: roles of medial prefrontal cortex and anterior cingulate cortex. *Behavioural Brain Research*, *250*, 91–101.

Bowles, B., Crupi, C., Mirsattari, S., Pigott, S., Parrent, A., Pruessner, J., … Köhler, S. (2007). Impaired familiarity with preserved recollection after anterior temporal-lobe resection that spares the hippocampus. *Proceedings of the National Academy of Sciences*, *104*(41), 16382–16387.

Brown, M., & Aggleton, J. (2001). Recognition memory: What are the roles of the perirhinal cortex and hippocampus? *Nature Reviews Neuroscience*, *2*(1), 51–61.

Browning, P. G., Baxter, M. G., & Gaffan, D. (2013). Prefrontal-temporal disconnection impairs recognition memory but not familiarity discrimination. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *33*(23), 9667–74.

Buffalo, E., Ramus, S., Clark, R., Teng, E., Squire, L., & Zola, S. (1999). Dissociation Between the Effects of Damage to Perirhinal Cortex and Area TE. *Learning & Memory*, *6*(6), 572–599.

Burke, S. N., Hartzell, A. L., Lister, J. P., Hoang, L. T., & Barnes, C. A. (2012). Layer V perirhinal cortical ensemble activity during object exploration: A comparison between young and aged rats. *Hippocampus*, *22*(10), 2080–2093.

Burnham, KE, Bannerman, DM, Dawson, LA, & Southam, E. (2010). Fos expression in the brains of rats performing an attentional set-shifting task. *Neuroscience*.

Bussey, T. J., Saksida, L. M., & Murray, E. A. (2002). Perirhinal cortex resolves feature ambiguity in complex visual discriminations. *The European Journal of Neuroscience*, *15*(2), 365–74.

Bussey, T. J., Saksida, L. M., & Murray, E. A. (2003). Impairments in visual discrimination after perirhinal cortex lesions: testing “declarative” vs. “perceptual-mnemonic” views of perirhinal cortex function. *The European Journal of Neuroscience*, *17*(3), 649–60.

Bussey, T. J., Saksida, L. M., & Murray, E. A. (2005). The perceptual-mnemonic/feature conjunction model of perirhinal cortex function. *The Quarterly Journal of Experimental Psychology. B, Comparative and Physiological Psychology*, *58*(3-4), 269–82.

Bussey, T. J., Saksida, L. M., & Murray, E. A. (2006). Perirhinal cortex and feature-ambiguous discriminations. *Learning & Memory (Cold Spring Harbor, N.Y.)*, *13*(2), 103–5; author reply 106–7.

Butter, C. M. (1969). Perseveration in extinction and in discrimination reversal tasks following selective frontal ablations in Macaca mulatta. *Physiology & Behavior*, *4*(2), 163-171.

Butterly, D., Petroccione, M., & Smith, D. (2012). Hippocampal context processing is critical for interference free recall of odor memories in rats. *Hippocampus*, *22*(4), 906–913.

Cannon, T., Glahn, D., Kim, J., Erp, T., Karlsgodt, K., Cohen, M., … Shirinyan, D. (2005). Dorsolateral prefrontal cortex activity during maintenance and manipulation of information in working memory in patients with schizophrenia. *Archives of General Psychiatry*, *62*(10), 1071–80.

Cavada, C., Tejedor, J., Cruz-Rizzolo, R. J., & Reinoso-Suárez, F. (2000). The anatomical connections of the macaque monkey orbitofrontal cortex. A review. *Cerebral Cortex*, *10*(3), 220–242.

Chlan-Fourney, J., Webster, M. J., Felleman, D. J., & Bachevalier, J. (2000). Neonatal medial temporal lobe lesions alter the distribution of tyrosine hydroxylase immunoreactive varicosities in the macaque prefrontal cortex (Vol. 228.18). Washington D.C.: Neuroscience Meeting Planner.

Cioni, G., D’Acunto, G., & Guzzetta, A. (2011). Perinatal brain damage in children: neuroplasticity, early intervention, and molecular mechanisms of recovery. *Progress in Brain Research*, *189*, 139–54.

Cohen, J. (1973). Eta-squared and partial eta-squared in fixed factor ANOVA designs. *Educational and Psychological Measurement*, *33*, 107–112.

Collins, P., Roberts, A. C., Dias, R., Everitt, B. J., & Robbins, T. W. (1998). Perseveration and strategy in a novel spatial self-ordered sequencing task for nonhuman primates: effects of excitotoxic lesions and dopamine depletions of the prefrontal cortex. *Journal of cognitive neuroscience*, *10*(3), 332-354.

Conklin, H., Luciana, M., Hooper, C., & Yarger, R. (2007). Working memory performance in typically developing children and adolescents: behavioral evidence of protracted frontal lobe development. *Developmental Neuropsychology*, *31*(1), 103–28.

Constantinidis, C., & Procyk, E. (2004). The primate working memory networks. *Cognitive, Affective & Behavioral Neuroscience*, *4*(4), 444–65.

Croxson, P. L., Johansen-Berg, H., Behrens, T. E., Robson, M. D., Pinsk, M. A., Gross, C. G., … Rushworth, M. F. (2005). Quantitative investigation of connections of the prefrontal cortex in the human and macaque using probabilistic diffusion tractography. *The Journal of Neuroscience*, *25*(39), 8854–8866.

D'Esposito, M., Postle, B., Ballard, D., & Lease, J. (1999). Maintenance versus Manipulation of Information Held in Working Memory: An Event-Related fMRI Study. *Brain and Cognition*, *41*(1), 6686

Davachi, L., & Goldman-Rakic, P. S. (2001). Primate rhinal cortex participates in both visual recognition and working memory tasks: functional mapping with 2-DG. *Journal of Neurophysiology*, *85*(6), 2590–2601.

Desimone, R., Schein, S. J., Moran, J., & Ungerleider, L. G. (1985). Contour, color and shape analysis beyond the striate cortex. *Vision Research*, *25*(3), 441–52.

Dew, I., & Cabeza, R. (2013). A Broader View of Perirhinal Function: From Recognition Memory to Fluency-Based Decisions. *The Journal of Neuroscience*, *33*(36), 14466–14474.

Diamond, A, Zola-Morgan, S, & Squire, LR. (1989). Successful performance by monkeys with lesions of the hippocampal formation on AB and object retrieval, two tasks that mark developmental changes in human infants. *Behavioral Neuroscience*, *103*(3), 526–537.

Dias, R., Robbins, T. W., & Roberts, A. C. (1997). Dissociable forms of inhibitory control within prefrontal cortex with an analog of the Wisconsin Card Sort Test: restriction to novel situations and independence from "on-line" processing. *Journal of Neuroscience*, *17*(23), 9285-9297.

Eacott, M., Gaffan, D., & Murray, E. (1994). Preserved Recognition Memory for Small Sets, and Impaired Stimulus Identification for Large Sets, Following Rhinal Cortex Ablations in Monkeys. *European Journal of Neuroscience*, *6*(9), 1466–1478.

Eichenbaum, H., Yonelinas, A. P., & Ranganath, C. (2007). The Medial Temporal Lobe and Recognition Memory. *Neuroscience*, *30*(1), 123–152.

Eldridge, L. L., Knowlton, B. J., Furmanski, C. S., Bookheimer, S. Y., & Engel, S. A. (2000). Remembering episodes: a selective role for the hippocampus during retrieval. *Nature neuroscience*, *3*(11), 1149-1152.

Erickson, CA, Jagadeesh, B, & Desimone, R. (2000). Clustering of perirhinal neurons with similar properties following visual experience in adult monkeys. *Nature Neuroscience*, *3*(11), 1143–1148.

Fuster, J. M. (2002). Frontal lobe and cognitive development. *Journal of Neurocytology*, *31*(2-5), 373–385.

Glahn, D., Kim, J., Cohen, M., Poutanen, V., Therman, S., Bava, S., … Cannon, T. (2002). Maintenance and Manipulation in Spatial Working Memory: Dissociations in the Prefrontal Cortex. *NeuroImage*, *17*(1), 201-213.

Glenn, M. J., Lehmann, H., Mumby, D. G., & Woodside, B. (2005). Differential fos expression

following aspiration, electrolytic, or excitotoxic lesions of the perirhinal cortex in rats. *Behavioral Neuroscience*, *119*(3), 806.

Godsil, B., Kiss, J., Spedding, M., & Jay, T. (2013). The hippocampal–prefrontal pathway: The weak link in psychiatric disorders? *European Neuropsychopharmacology*, *23*(10).

Goldman, PS, & Rosvold, HE. (1970). Localization of function within the dorsolateral prefrontal cortex of the rhesus monkey. *Experimental Neurology*.

Goldman, P. S., Rosvold, H. E., & Mishkin, M. (1970). Selective sparing of function following prefrontal lobectomy in infant monkeys. *Experimental Neurology*, *29*(2), 221–6.

Goldman-Rakic, P. S., Selemon, L. D., & Schwartz, M. L. (1984). Dual pathways connecting the dorsolateral prefrontal cortex with the hippocampal formation and parahippocampal cortex in the rhesus monkey. *Neuroscience*, *12*(3), 719–43.

Goursaud, A., & Bachevalier, J. (2007). Social attachment in juvenile monkeys with neonatal lesion of the hippocampus, amygdala and orbital frontal cortex. *Behavioural Brain Research*, *176*(1), 75–93.

Guedj, E., Barbeau, E., Liégeois-Chauvel, C., Confort-Gouny, S., Bartolomei, F., Chauvel, P., … Guye, M. (2010). Performance in recognition memory is correlated with entorhinal/perirhinal interictal metabolism in temporal lobe epilepsy. *Epilepsy & Behavior*, *19*(4), 612–7.

Hales, J. B., Broadbent, N. J., Velu, P. D., Squire, L. R., & Clark, R. E. (2015). Hippocampus, perirhinal cortex, and complex visual discriminations in rats and humans. *Learning & Memory*, *22*(2), 83–91.

Hampton, R. R. (2005). Monkey perirhinal cortex is critical for visual memory, but not for visual perception: reexamination of the behavioural evidence from monkeys. *The Quarterly Journal of Experimental Psychology. B, Comparative and Physiological Psychology*, *58*(3-4), 283–99.

Hampton, R. R., & Murray, E. A. (2002). Learning of discriminations is impaired, but generalization to altered views is intact, in monkeys (Macaca mulatta) with perirhinal cortex removal. *Behavioral Neuroscience*, *116*(3), 363–77.

Haxby, J. V., Grady, C. L., Horwitz, B., Ungerleider, L. G., Mishkin, M., Carson, R. E., … Rapoport, S. I. (1991). Dissociation of object and spatial visual processing pathways in human extrastriate cortex. *Proceedings of the National Academy of Sciences of the United States of America*, *88*(5), 1621–5.

Heuer, E., & Bachevalier, J. (2011a). Effects of selective neonatal hippocampal lesions on tests of object and spatial recognition memory in monkeys. *Behavioral Neuroscience*, *125*(2), 137–49.

Heuer, E., & Bachevalier, J. (2011b). Neonatal hippocampal lesions in rhesus macaques alter the monitoring, but not maintenance, of information in working memory. *Behavioral Neuroscience*, *125*(6), 859–70. 41

Heuer, E., & Bachevalier, J. (2013). Working memory for temporal order is impaired after selective neonatal hippocampal lesions in adult rhesus macaques. *Behavioural Brain Research*, *239*, 55–62.

Insausti, R., Amaral, D., & Cowan, W. (1987). The entorhinal cortex of the monkey: II. Cortical afferents. *Journal of Comparative Neurology*, *264*(3), 356–395.

Iversen, S. D., & Mishkin, M. (1970). Perseverative interference in monkeys following selective lesions of the inferior prefrontal convexity. *Experimental Brain Research*, *11*(4), 376–86.

Jabès, A., Lavenex, P., Amaral, D., & Lavenex, P. (2010). Quantitative analysis of postnatal neurogenesis and neuron number in the macaque monkey dentate gyrus. *European Journal of Neuroscience*, *31*(2), 273–285.

Jabès, A., Lavenex, P., Amaral, D., & Lavenex, P. (2011). Postnatal development of the hippocampal

formation: A stereological study in macaque monkeys. *The Journal of Comparative Neurology*, *519*(6), 1051–1070.

Jeneson, A., & Squire, L. (2012). Working memory, long-term memory, and medial temporal lobe function. *Learning & Memory*, *19*(1), 15–25.

Jha, A. P., Fabian, S. A., & Aguirre, G. K. (2004). The role of prefrontal cortex in resolving distractor interference. *Cognitive, Affective & Behavioral Neuroscience*, *4*(4), 517–27.

Kazama, A., Davis, M., & Bachevalier, J. (2014). Neonatal lesions of orbital frontal areas 11/13 in monkeys alter goal-directed behavior but spare fear conditioning and safety signal learning. *Frontiers in Neuroscience*, *8*.

Keppel, G., & Wickens, T. D. (2004). *Design and Analysis* (4th ed., p. 612). Upper Saddle River, New Jersey 07458: Pearson.

Kimble, D. P., & Pribram, K. H. (1963). Hippocampectomy and behavior sequences. *Science*, *139*(3557), 824–825.

Kolb, B., Mychasiuk, R., Muhammad, A., & Gibb, R. (2013). Brain plasticity in the developing brain. *Prog Brain Res*, *207*, 35-64.

Kolb, B., & Gibb, R. (2007). Brain plasticity and recovery from early cortical injury. *Developmental psychobiology*, *49*(2), 107-118.

Kolb, B., & Gibb, R. (2011). Brain plasticity and behaviour in the developing brain. *Journal of the Canadian Academy of Child and Adolescent Psychiatry*, *20*(4), 265–76.

Kondo, H., Saleem, K., & Price, J. (2005). Differential connections of the perirhinal and parahippocampal cortex with the orbital and medial prefrontal networks in macaque monkeys. *Journal of Comparative Neurology*, *493*(4), 479–509.

Kowalska, D. M., Bachevalier, J., & Mishkin, M. (1991). The role of the inferior prefrontal convexity in performance of delayed nonmatching-to-sample. *Neuropsychologia*, *29*(6), 583–600.

Lavenex, P., Suzuki, W., & Amaral, D. (2002). Perirhinal and parahippocampal cortices of the macaque monkey: Projections to the neocortex. *Journal of Comparative Neurology*, *447*(4), 394–420.

Lavenex, P., Suzuki, W., & Amaral, D. (2004). Perirhinal and parahippocampal cortices of the macaque monkey: Intrinsic projections and interconnections. *Journal of Comparative Neurology*, *472*(3), 371–394.

Lee, A., Buckley, M., Gaffan, D., Emery, T., Hodges, J., & Graham, K. (2006). Differentiating the Roles of the Hippocampus and Perirhinal Cortex in Processes beyond Long-Term Declarative Memory: A Double Dissociation in Dementia. *The Journal of Neuroscience*, *26*(19), 5198–5203.

Lehky, S., & Tanaka, K. (2007). Enhancement of Object Representations in Primate Perirhinal Cortex During a Visual Working-Memory Task. *Journal of Neurophysiology*, *97*(2), 1298–1310.

Levine, T., & Hullett, C. (2002). Eta Squared, Partial Eta Squared, and Misreporting of Effect Size in Communication Research. *Human Communication Research*, *28*(4), 612–625.

Libby, L., Ekstrom, A., Ragland, J., & Ranganath, C. (2012). Differential Connectivity of Perirhinal and Parahippocampal Cortices within Human Hippocampal Subregions Revealed by High-Resolution Functional Imaging. *The Journal of Neuroscience*, *32*(19), 6550–6560.

Liu, Z. I., & Richmond, B. J. (2000). Response differences in monkey TE and perirhinal cortex: stimulus association related to reward schedules. *Journal of Neurophysiology*, *83*(3), 1677–1692.

Machado, C. J., & Bachevalier, J. (2003). Non-human primate models of childhood psychopathology: the promise and the limitations. *Journal of Child Psychology and Psychiatry*, *44*(1), 64-87.

Machado, C. J., & Nelson, E. E. (2011). Eye tracking with nonhuman primates is now more accessible

than ever before. *American Journal of Primatology*, *73*(6), 562–569.

Malkova, L., Alvarado, M. C., & Bachevalier, J. (2014). Effects of Separate or Combined Neonatal Damage to the Orbital Frontal Cortex and the Inferior Convexity on Object Recognition in Monkeys. *Cerebral Cortex*.

Málková, L., Bachevalier, J., Mishkin, M., & Saunders, R. C. (2001). Neurotoxic lesions of perirhinal cortex impair visual recognition memory in rhesus monkeys. *Neuroreport*, *12*(9), 1913–7.

Malkova, L., Bachevalier, J., Webster, M., & Mishkin, M. (2000). Effects of Neonatal Inferior Prefrontal and Medial Temporal Lesions on Learning the Rule for Delayed Nonmatching-to-Sample. *Developmental Neuropsychology*, *18*(3), 399–421.

Málková, L., Lex, C. K., Mishkin, M., & Saunders, R. C. (2001). MRI-based evaluation of locus and extent of neurotoxic lesions in monkeys . *Hippocampus*, *11*(4), 361–70.

Meng, Y., Li, L., Hu, X., Bachevalier, J., Payne, C., & Zhang, X. (2013). Differential alterations of dorsolateral and ventrolateral prefrontal cortex in adult macaques with neonatal hippocampal lesion: A diffusion Tensor Imaging study. Abstract presented at International Society for Magnetic Resonance in Medicine. Salt Lake City, UT, US.

Meunier, M., Bachevalier, J., & Mishkin, M. (1997). Effects of orbital frontal and anterior cingulate lesions on object and spatial memory in rhesus monkeys. *Neuropsychologia*, *35*(7), 999–1015.

Meunier, M., Bachevalier, J., Mishkin, M., & Murray, E. A. (1993). Effects on visual recognition of combined and separate ablations of the entorhinal and perirhinal cortex in rhesus monkeys. *The Journal of Neuroscience* , *13*(12), 5418–5432.

Meunier, M., Bachevalier, J., Murray, E. A., Málková, L., & Mishkin, M. (1999). Effects of aspiration versus neurotoxic lesions of the amygdala on emotional responses in monkeys. *The European Journal of Neuroscience*, *11*(12), 4403–18.

Meunier, M., Hadfield, W., Bachevalier, J., & Murray, E. A. (1996). Effects of rhinal cortex lesions combined with hippocampectomy on visual recognition memory in rhesus monkeys. *Journal of Neurophysiology*, *75*(3), 1190–1205.

Miller, E. A., Goldman, P. S., & Rosvold, H. E. (1973). Delayed Recovery of Function follwing Orbital Prefrontal Lesions in Infant Monkeys. *Science*, *182*(4109), 304-306.

Miller, E. K., & Buschman, T. J. (2013). Cortical circuits for the control of attention. *Current Opinion in Neurobiology*, *23*(2), 216–22.

Miller, E. K., & Cohen, J. (2003). An integrative theory of prefrontal cortical function. *Neuroscience*, *24*(1), 167–202.

Mishkin, M. (1978). Memory in monkeys severely impaired by combined but not by separate removal of amygdala and hippocampus. *Nature*, *273*(5660), 297–298.

Mishkin, M., & Manning, F. J. (1978). Non-spatial memory after selective prefrontal lesions in monkeys. *Brain Research*, *143*(2), 313–23.

Mishkin, M., & Ungerleider, L. G. (1982). Contribution of striate inputs to the visuospatial functions of parieto-preoccipital cortex in monkeys. *Behavioural Brain Research*, *6*(1), 57–77.

Monchi, O., Petrides, M., Petre, V., Worsley, K., & Dagher, A. (2001). Wisconsin Card Sorting revisited: distinct neural circuits participating in different stages of the task identified by event-related functional magnetic resonance imaging. *The Journal of Neuroscience*, *21*(19), 7733–41.

Morris, R., Pandya, D. N., & Petrides, M. (1999). Morris, R., Pandya, D. N., & Petrides, M. (1999). Fiber system linking the mid-dorsolateral frontal cortex with the retrosplenial/presubicular region in the rhesus monkey. *Journal of Comparative Neurology*, *407*(2), 183–192.

Mumby, D. G., & Pinel, J. P. (1994). Rhinal cortex lesions and object recognition in rats. *Behavioral*

Neuroscience, *108*(1), 11.

Murray, E. A., Gaffan, D., & Mishkin, M. (1993). Neural substrates of visual stimulus-stimulus association in rhesus monkeys. *Journal of Neuroscience*, *13*(10), 4549-4561.

Murray, E. A., Baxter, M. G., & Gaffan, D. (1998). Monkeys with rhinal cortex damage or neurotoxic hippocampal lesions are impaired on spatial scene learning and object reversals. *Behavioral Neuroscience*, *112*(6), 1291.

Murray, E. A., & Mishkin, M. (1998). Object recognition and location memory in monkeys with excitotoxic lesions of the amygdala and hippocampus. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *18*(16), 6568–82.

Murray, E., & Wise, S. (2012). Why is there a special issue on perirhinal cortex in a journal called hippocampus? The perirhinal cortex in historical perspective. *Hippocampus*, *22*(10), 1941–1951.

Murray, & Richmond, B. J. (2001). Role of perirhinal cortex in object perception, memory, and associations. *Current Opinion in Neurobiology*, *11*(2), 188–93.

National Research Council (US). (2011). *Guide for the Care and Use of Laboratory Animals* (8th edition). Washington (DC): National Academies Press.

Nemanic, S., Alvarado, M. C., & Bachevalier, J. (2004). The hippocampal/parahippocampal regions and recognition memory: insights from visual paired comparison versus object-delayed nonmatching in monkeys. *The Journal of Neuroscience*, *24*(8), 2013–26.

Nemanic, S., Alvarado, M. C., Price, R. E., Jackson, E. F., & Bachevalier, J. (2002). Assessment of locus and extent of neurotoxic lesions in monkeys using neuroimaging techniques: a replication. *Journal of Neuroscience Methods*, *121*(2), 199–209.

Owen, A. M., Herrod, N. J., Menon, D. K., Clark, J. C., Downey, S. P., Carpenter, T. A., ... & Sahakian, B. J. (1999). Redefining the functional organization of working memory processes within human lateral prefrontal cortex. *European Journal of Neuroscience*, *11*(2), 567-574.

Owen, A. M., Roberts, A. C., Polkey, C. E., Sahakian, B. J., & Robbins, T. W. (1991). Extra-dimensional versus intra-dimensional set shifting performance following frontal lobe excisions, temporal lobe excisions or amygdalo-hippocampectomy in man. *Neuropsychologia*, *29*(10), 993–1006.

Owen, A. M., Roberts, A. C., Hodges, J. R., & Robbins, T. W. (1993). Contrasting mechanisms of impaired attentional set-shifting in patients with frontal lobe damage or Parkinson's disease. *Brain*, *116*(5), 1159-1175.

Passingham, R. (1975). Delayed matching after selective prefrontal lesions in monkeys (Macaca mulatta). *Brain research*, *92*(1), 89-102.

Payne, C., Machado, C., Bliwise, N., & Bachevalier, J. (2010). Maturation of the hippocampal formation and amygdala in Macaca mulatta: A volumetric magnetic resonance imaging study. *Hippocampus*, *20*(8), 922–935.

Petrides, M. (1991a). Functional Specialization within the Dorsolateral Frontal Cortex for Serial Order Memory. *Proceedings of the Royal Society B: Biological Sciences*, *246*(1317), 299-306.

Petrides, M. (1991b). Monitoring of Selections of Visual Stimuli and the Primate Frontal Cortex. *Proceedings of the Royal Society B: Biological Sciences*, *246*(1317), 293-298.

Petrides, M. (1995). Impairments on nonspatial self-ordered and externally ordered working memory tasks after lesions of the mid-dorsal part of the lateral frontal cortex in the monkey. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *15*(1 Pt 1), 359–75.

Petrides, M. (2000). The role of the mid-dorsolateral prefrontal cortex in working memory. In *Executive Control and the Frontal Lobe: Current Issues* (pp. 44-54). Springer Berlin Heidelberg.

Petrides, M., & Milner, B. (1982). Deficits on subject-ordered tasks after frontal-and temporal-lobe lesions in man. *Neuropsychologia*, *20*(3), 249-262.

Petrides, M., & Pandya, D. (2002). Comparative cytoarchitectonic analysis of the human and the macaque ventrolateral prefrontal cortex and corticocortical connection patterns in the monkey. *European Journal of Neuroscience*, *16*(2), 291–310.

Pitkänen, A., & Amaral, D. G. (1993). Distribution of parvalbumin-immunoreactive cells and fibers in the monkey temporal lobe: The hippocampal formation. *Journal of Comparative Neurology*, *331*(1), 37-74.

Postle, B. R., Brush, L. N., & Nick, A. M. (2004). Prefrontal cortex and the mediation of proactive interference in working memory. *Cognitive, Affective & Behavioral Neuroscience*, *4*(4), 600–8.

Ranganath, C. (2006). Working memory for visual objects: Complementary roles of inferior temporal, medial temporal, and prefrontal cortex. *Neuroscience*, *139*(1).

Ranganath, C., Cohen, M. X., Dam, C., & D'Esposito, M. (2004). Inferior temporal, prefrontal, and hippocampal contributions to visual working memory maintenance and associative memory retrieval. *The Journal of Neuroscience*, *24*(16), 3917–3925.

Raper, J., Bachevalier, J., Wallen, K., & Sanchez, M. (2013). Neonatal amygdala lesions alter basal cortisol levels in infant rhesus monkeys. *Psychoneuroendocrinology*, *38*(6), 818-829.

Raper, J., Wilson, M., Sanchez, M., Machado, C. J., & Bachevalier, J. (2013). Pervasive alterations of emotional and neuroendocrine responses to an acute stressor after neonatal amygdala lesions in rhesus monkeys. *Psychoneuroendocrinology*, *38*(7), 1021-1035.

Richmond, J., Sowerby, P., Colombo, M., & Hayne, H. (2004). The effect of familiarization time, retention interval, and context change on adults' performance in the visual paired comparison task. *Developmental Psychobiology*, *44*(2), 146–155.

Ridderinkhof, K. R., Span, M., & Molen, M. (2002). Perseverative Behavior and Adaptive Control in Older Adults: Performance Monitoring, Rule Induction, and Set Shifting. *Brain and Cognition*, *49*(3), 382–401.

Roberts, A., Robbins, T., & Everitt, B. (1988). The effects of intradimensional and extradimensional shifts on visual discrimination learning in humans and non-human primates. *The Quarterly Journal of Experimental Psychology Section B: Comparative and Physiological Psychology*, *40*(4), 321–341.

Rogers, R. D., Andrews, T. C., Grasby, P. M., Brooks, D. J., & Robbins, T. W. (2000). Contrasting cortical and subcortical activations produced by attentional-set shifting and reversal learning in humans. *Journal of Cognitive Neuroscience*, *12*(1), 142–62.

Rosnow, R., & Rosenthal, R. (1996). Computing contrasts, effect sizes, and counternulls on other people's published data: General procedures for research consumers. *Psychological Methods*, *1*(4), 331.

Ross, T. P., Hanouskova, E., Giarla, K., Calhoun, E., & Tucker, M. (2007). The reliability and validity of the self-ordered pointing task. *Archives of Clinical Neuropsychology*, *22*(4), 449–58.

Sahakian, B. J., & Owen, A. M. (1992). Computerized assessment in neuropsychiatry using CANTAB: discussion paper. *Journal of the Royal Society of Medicine*, *85*(7), 399.

Saunders, R., Mishkin, M., & Aggleton, J. (2005). Projections from the entorhinal cortex, perirhinal cortex, presubiculum, and parasubiculum to the medial thalamus in macaque monkeys: identifying different pathways using disconnection techniques. *Experimental Brain Research*, *167*(1), 1–16.

Schoemaker, D., Gauthier, S., & Pruessner, J. (2014). Recollection and Familiarity in Aging Individuals with Mild Cognitive Impairment and Alzheimer's Disease: A Literature Review.

Neuropsychology Review, *24*(3), 313-331.

Scoville, W., & Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. *Journal of Neurology, Neurosurgery & Psychiatry*, *20*(1), 11–21.

Shapiro, R. C., & Olton, D. S. (1994). Hippocampal function and interference. (pp. 141–146). London: MIT.

Staresina, B., Fell, J., Lam, A., Axmacher, N., & Henson, R. (2012). Memory signals are temporally dissociated in and across human hippocampus and perirhinal cortex. *Nature Neuroscience*, *15*(8), 1167–1173.

Staresina, B. P., Fell, J., Dunn, J. C., Axmacher, N., & Henson, R. N. (2013). Using state-trace analysis to dissociate the functions of the human hippocampus and perirhinal cortex in recognition memory. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(8), 3119–24.

Stern, C., Sherman, S., Kirchhoff, B., & Hasselmo, M. (2001). Medial temporal and prefrontal contributions to working memory tasks with novel and familiar stimuli. *Hippocampus*, *11*(4), 337–346.

Suzuki, W. (1996a). Neuroanatomy of the monkey entorhinal, perirhinal and parahippocampal cortices: organization of cortical inputs and interconnections with amygdala and striatum, *Seminars in Neuroscience 8*(1), 3–12.

Suzuki, W. A. (1996b). The anatomy, physiology and functions of the perirhinal cortex. *Current opinion in neurobiology*, *6*(2), 179-186.

Suzuki, W. A., & Amaral, D. G. (1994a). Perirhinal and parahippocampal cortices of the macaque monkey: cortical afferents. *Journal of comparative neurology*, *350*(4), 497-533.

Suzuki, W. A., & Amaral, D. G. (1994b). Topographic organization of the reciprocal connections between the monkey entorhinal cortex and the perirhinal and parahippocampal cortices. *Journal of Neuroscience*, *14*(3), 1856-1877.

Suzuki, W., & Naya, Y. (2014). The perirhinal cortex. *Neuroscience*, *37*(1), 39–53.

Suzuki, W., Zola-Morgan, S., Squire, L., & Amaral, D. (1993). Lesions of the perirhinal and parahippocampal cortices in the monkey produce long-lasting memory impairment in the visual and tactual modalities. *The Journal of Neuroscience*, *13*(6), 2430–2451.

Takesian, A. E., & Hensch, T. K. (2013). Balancing plasticity/stability across brain development. *Prog Brain Res*, *207*, 3-34.

Thornton, J. A., Rothblat, L. A., & Murray, E. A. (1997). Rhinal cortex removal produces amnesia for preoperatively learned discrimination problems but fails to disrupt postoperative acquisition and retention in rhesus monkeys. *Journal of Neuroscience*, *17*(21), 8536-8549.

Tseng, K., Chambers, A., & Lipska, B. (2009). The neonatal ventral hippocampal lesion as a heuristic neurodevelopmental model of schizophrenia. *Behavioural Brain Research*, *204*(2), 295–305.

Tseng, K., Lewis, B., Hashimoto, T., Sesack, S., Kloc, M., Lewis, D., & O'Donnell, P. (2008). A Neonatal Ventral Hippocampal Lesion Causes Functional Deficits in Adult Prefrontal Cortical Interneurons. *The Journal of Neuroscience*, *28*(48), 12691–12699.

Tu, H.-W. W., Hampton, R. R., & Murray, E. A. (2011). Perirhinal cortex removal dissociates two memory systems in matching-to-sample performance in rhesus monkeys. *The Journal of Neuroscience*, *31*(45), 16336–43.

Tunon, T., Insausti, R., Ferrer, I., Sobreviela, T., & Soriano, E. (1992). Parvalbumin and calbindin D-28K in the human entorhinal cortex. An immunohistochemical study. *Brain research*, *589*(1), 24-32.

Ungerleider, L. G., & Haxby, J. V. (1994). "What" and "where" in the human brain. *Current Opinion in Neurobiology*, *4*(2), 157–65.

Vilberg, K. L., & Davachi, L. (2013). Perirhinal-Hippocampal Connectivity during Reactivation Is a Marker for Object-Based Memory Consolidation. *Neuron*, *79*(6), 1232–1242.

Wan, H., Aggleton, J. P., & Brown, M. W. (1999). Different contributions of the hippocampus and perirhinal cortex to recognition memory. *The Journal of Neuroscience*, *19*(3), 1142–1148.

Warburton, C., & Brown, M. (2010). Findings from animals concerning when interactions between perirhinal cortex, hippocampus and medial prefrontal cortex are necessary for recognition memory. *Neuropsychologia*, *48*(8).

Warburton, E. C., & Brown, M. W. (2014). Neural circuitry for rat recognition memory. *Behavioural Brain Research*. 285, 131-139.

Weiss, A., & Bachevalier, J. (2016). Object and spatial memory after neonatal perirhinal lesions in monkeys. *Behavioural Brain Research*, *298*(Pt B), 210–217.

Weiss, AR. (unpublished masters thesis). *Is the perirhinal cortex involved in working memory?* 2014 Emory University, Atlanta, GA.

Weiss, Nadji, R., & Bachevalier, J. (2016). Neonatal Perirhinal Lesions in Rhesus Macaques Alter Performance on Working Memory Tasks with High Proactive Interference. *Frontiers in Systems Neuroscience*, *9*.

Wilson, H. R., & Wilkinson, F. (2015). From orientations to objects: Configural processing in the ventral stream. *Journal of Vision*, *15*(7), 4.

Yonelinas, A. P., Kroll, N. E., Quamme, J. R., Lazzara, M. M., Sauvé, M.-J. J., Widaman, K. F., & Knight, R. T. (2002). Effects of extensive temporal lobe damage or mild hypoxia on recollection and familiarity. *Nature Neuroscience*, *5*(11), 1236–41.

Zeamer, A., & Bachevalier, J. (2013). Long-term effects of neonatal hippocampal lesions on novelty preference in monkeys. *Hippocampus*, *23*(9), 745–750.

Zeamer, A., Heuer, E., & Bachevalier, J. (2010). Developmental Trajectory of Object Recognition Memory in Infant Rhesus Macaques with and without Neonatal Hippocampal Lesions. *The Journal of Neuroscience*, *30*(27), 9157–9165.

Zeamer, A., Meunier, M., & Bachevalier, J. (2011). Stimulus similarity and encoding time influence incidental recognition memory in adult monkeys with selective hippocampal lesions. *Learning & Memory (Cold Spring Harbor, N.Y.)*, *18*(3), 170–80.

Zeamer, A., Richardson, R. L., Weiss, A. R., & Bachevalier, J. (2015). The development of object recognition memory in rhesus macaques with neonatal lesions of the perirhinal cortex. *Developmental Cognitive Neuroscience*, *11*, 31–41.

Zhu, X. O., McCabe, B. J., Aggleton, J. P., & Brown, M. W. (1997). Differential activation of the rat hippocampus and perirhinal cortex by novel visual stimuli and a novel environment. *Neuroscience Letters*, *229*(2), 141–143.

Zola-Morgan, S., Squire, L. R., & Amaral, D. G. (1989). Lesions of the amygdala that spare adjacent cortical regions do not impair memory or exacerbate the impairment following lesions of the hippocampal formation. *The Journal of Neuroscience*, *9*(6), 1922–1936.

Zola-Morgan, & Squire, L. R. (1985). Medial temporal lesions in monkeys impair memory on a variety of tasks sensitive to human amnesia. *Behavioral Neuroscience*, *99*(1), 22–34.