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Jessica White April 11, 2016

Reversal Learning in Rhesus Macaques is impaired after Neonatal Perirhinal Lesions

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Abstract

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Neonatal damage to the perirhinal (Neo-PRh) cortex in rhesus macaques impaired performance on working memory tasks with high proactive interference. To determine if this inability to overcome proactive interference was due to impaired stimulus-reward association learning or impaired behavioral flexibility, the same rhesus macaques (Neo-PRh) and age-matched sham-operated controls (Neo-C) were tested using an abbreviated version of the Intradimensional/Extradimensional (ID/ED) Set Shifting task. The task consisted of successively acquiring two simple discrimination problems using colored shape stimuli, followed by three serial reversals requiring behavioral flexibility. Finally, a complex discrimination stage was given in which responses to the shape stimuli had to be maintained in the presence of a new set of line stimuli overlaid on the original shape stimuli. Adult monkeys with Neo-PRh lesions performed as well as control monkeys in all discrimination stages, but were impaired on the serial reversals. These findings indicate that neonatal PRh lesions in monkeys impaired the use of behavioral flexibility, but spared stimulus-reward association learning. Although this study confirmed an impairment in behavioral flexibility as a result of neonatal PRh lesions that may be at the source of their inability to overcome proactive interference, we cannot rule out that impaired performance in working memory tasks with high proactive interference might have also resulted from impaired cognitive flexibility. Future work will need to test this possibility by continuing the training the same Neo-PRh monkeys on the extradimensional shift (EDS) stage of the ID/ED task. In this stage, the previously attended stimuli (shapes) must be ignored and, instead, the previously ignored stimuli (lines) must be attended. This shift in attentional set requires the use of cognitive flexibility that might be essential to overcome proactive interference in working memory tasks.

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Reversal Learning in Rhesus Macaques is impaired after Neonatal Perirhinal Lesions

Jessica White

Introduction

Learning relies on certain mental processes that facilitate the memory of new information. In some cases, learning new information is disrupted by the retention of old information, a phenomenon known as proactive interference (Crannell, 1948). Proactive interference accumulates on tasks that require participants to suppress responding based on previous memories, and instead base responses on their most-current experiences. Many of these tasks present the same stimuli to participants for every trial, but change the reinforcement contingencies associated with each stimulus. For example, in reversal learning tasks, previously learned rules are switched and must be re-learned. This capacity requires the ability to resolve proactive interference and is considered to be a measure of flexible behavior (Mala et al., 2015). An inability to overcome proactive interference on working memory tasks that re-use the same stimuli across trials, and in reversal tasks, is observed in clinical populations with prefrontal cortex damage, Parkinson's disease, and Autism Spectrum Disorders (Stuss et al., 1982, Gauntlett-Gilbert et al., 1999, Yerys et al., 2009), suggesting that these disorders share a common impairment in behavioral flexibility. Studies that promote a deeper understanding of the mechanisms important to overcome proactive interference will aid in the development of new therapies for diseases with impairments in behavioral flexibility. The current study aims to build on previous findings that monkeys with neonatal perirhinal lesions are impaired on working memory tasks that generate proactive interference by examining their performance on a task that involves stimulus-response association learning and behavioral flexibility, the truncated Intradimensional/Extradimensional (ID/ED) Set Shifting task.

Working memory refers to temporary representations of recently gathered or recalled information (D'Esposito, 2007). One proposed model of working memory posits that a central executive entity directs the attention of working memory processes to the phonological loop and visuospatial sketchpad subsystems, which are responsible for phonological information and visuospatial information, respectively (Baddeley, 1974). This model led to further studies that suggested that working memory involves different processes, i.e. maintenance, monitoring, and manipulation of information and have identified the prefrontal cortex (PFC) as a structure specifically critical to support working memory (Goldman-Rakic, 1990, Petrides, 1995, Courtney et al., 1998). Maintenance and monitoring processes refer to keeping the information active in mind and monitoring refers to not only maintaining the exact content of the information, but also monitoring the order in which information was presented. Manipulation process is the ability to perform mental alterations of that information, for example performing mathematical operations on numbers held in mind. Previous research on working memory in humans revealed a correlation between the different working memory processes and activity in specific regions of the PFC (D'Esposito et al., 1999). Specifically, neuroimaging research indicates that when subjects perform a working memory task, the ventrolateral prefrontal cortex (VLPFC) is activated when subjects are asked to simply maintain the representation of a stimulus in memory, whereas the dorsolateral prefrontal cortex (DLPFC) is activated when subjects are asked to monitor and/or manipulate that information (D'Esposito et al., 1999). These results indicate the importance of the VLPFC for the maintenance processes and the DLPFC for the monitoring and manipulation processes of working memory. This same division of working memory processes within the PFC has also been shown in non-human primate models (Petrides, 1995). In monkeys, selective lesions of the DLPFC impair performance on working memory tasks involving

monitoring (Constantinidis and Procyk, 2004). Yet, recent evidence has indicated that working memory processes may be mediated by brain structures other than the PFC. Thus, functional neuroimaging studies in humans have indicated that working memory requires the interaction of a broader network of brain areas, including structures of the medial temporal lobe (MTL)(Constantinidis and Procyk, 2004, Libby et al., 2014).

The medial temporal lobe includes the hippocampus (H), perirhinal cortex (PRH), entorhinal cortex, and parahippocampal cortex, all of which are activated when human subjects performed a maintenance working memory task (Libby et al., 2014). Furthermore, the role of the hippocampus in working memory was investigated in earlier studies by Heuer and Bachevalier (2011) while studying the long-term effects of early damage to the hippocampus on memory processes in nonhuman primates. In this later study, working memory processes were measured in groups of adult monkeys that had received either neonatal hippocampal lesions (Neo-H) or sham-operations (Neo-C). The three working memory tasks included the Session-Unique Delayed Nonmatch- to- Sample task (SU-DNMS), the Object Self-Ordered task (Obj-SO), and the Self-Ordered Memory Task (SOMT). In the SU-DNMS task, which only tests maintenance working memory process, each trial consisted of two phases. In the first phase, monkeys were presented with a single object that they displaced to retrieve a reward. After a short 5s delay, the second phase consisted of presenting two objects to the monkey, i.e. the previously presented object and a second object that hides the reward. After a 30s intertrial interval, the same two objects were presented for each remaining trial of that day, with each object alternating as the sample object on each trial using a pseudorandom sequence. During the Obj-SO task, which tests both maintenance and monitoring working memory processes, monkeys were first presented with three objects, positioned horizontally in front of the monkey and each covering a reward hidden

in a well. The monkey was allowed to displace one of the three objects. After a short delay, the position of the three objects was shuffled but only the two unselected objects were rewarded. Following another short delay, the position of the objects was again shuffled but only the remaining unselected object was rewarded. The same objects were used for all testing sessions. Thus, the monkey had to monitor the selection that was made on previous choices to select a rewarded object. During the SOMT, the monkey was first presented with a list of objects one at a time, and then presented with two objects from the list. The monkey was rewarded for choosing the object that occurred earliest in the list. One version of this task uses three objects while another version uses four objects. The four-object SOMT requires the ability to remember the temporal order of the two middle objects as opposed to the three object SOMT where there is only one middle object. Impairment in identifying the temporal order of the middle two objects in the four object SOMT is associated with impaired monitoring of working memory and has been observed in monkeys with lesions to the DLPFC (Petrides, 1991). Neo-H monkeys performed as well as Neo-C monkeys in the SU-DNMS task, but were impaired in the Obj-SO task and in the four-object SOMT when discriminating the temporal order of the middle objects. The results from these studies indicated that neonatal lesions to the hippocampus (Neo-H) resulted in impaired monitoring of working memory, but spared maintenance processes. Since the DLPFC had previously been implicated in monitoring working memory processes (D'Esposito et al., 1999), this pattern of results suggested that the Neo-H lesions may have disrupted the development of the DLPFC but not the development of the VLPFC. Alternatively, given the recent demonstration that the hippocampus contributes to working memory processes in adulthood (Mitchell et al., 2000, Ranganath and D'Esposito, 2001, Piekema et al., 2006), the

impairment following the Neo-H lesions could have also resulted from selective damage to the hippocampus itself.

Although this later study provided evidence that the hippocampus can play a critical role in working memory processes, another MTL area that could contribute significantly to working memory is the perirhinal cortex (PRh). Indeed, as compared to the hippocampus that is only indirectly connected to PFC regions (Cavada et al., 2000, Croxson et al., 2005), the PRh has direct anatomical connections with the lateral prefrontal cortex (Suzuki and Amaral, 1994, Lavenex et al., 2002, Hirata et al., 2013) and may thus directly interact with the PFC to support working memory process (See Figure 1). To test this proposal, a more recent study by Weiss, Nadji & Bachevalier (2015) investigated the role of the PRh in working memory. A group of monkeys with neonatal lesions to the PRh (Neo-PRh) as well as control animals performed the same three working memory tasks as those in (Heuer and Bachevalier, 2011, 2013). These tests included the SU-DNMS task, Obj-SO task, and SOMT

The SU-DNMS task in this study had the same methods as in the previous study by Heuer and Bachevalier (2011). Because the SU-DNMS task uses the same two objects for each trial in one daily session, novelty can only be used to guide the response on the first trial. Thereafter, as the trials progress, the two objects become highly familiar and animals have to guide their choices based on the object they have seen the most recently (recency memory). Thus, this task generates proactive interference as trials progress. The Neo-PRh subjects were only transiently impaired on this task, i.e. they performed significantly worse than controls during the 5s short delay phase, but performed as well as controls when re-tested using longer, 30s, delays. The unimpaired performance of the Neo-PRh groups when the task demands were increased with the

30s delay indicates that these animals were able to maintain object representation and thus showed normal working memory.

The Obj-SO task also uses the same methods as those described in Heuer and Bachevalier (2011). In the Obj-SO task the same 3 objects are also used daily for each testing session and thus generate proactive interference across trials and testing sessions. The Neo-PRh subjects made significantly more perseverative errors compared to control animals. The impairments of the Neo-PRh animals in the Obj-SO task could have been caused by impaired working memory processes, however, an alternative explanation could be that these deficits were caused by proactive interference due to the repeated use of objects. In order to test this alternative explanation, the researchers used a task that requires working memory but does not generate proactive interference

To investigate whether the Neo-PRh group was indeed impaired in monitoring information in working memory, the researchers included an additional task, the SOMT. The SOMT also assesses both maintenance and monitoring processes but differed from SU-DNMS and Obj-SO in that a pool of novel objects is used for each trial of the task. The use of novel objects for each trial minimizes the impact of proactive interference on performance of the SOMT. The Neo-PRh monkeys were unimpaired in identifying the earlier-occurring object, indicating normal ability to monitor the temporal order of items.

Results from all three tasks indicated that, as compared to controls, animals with Neo-PRh lesions were impaired in working memory tasks only if the task involved high interference, as in the SU-DNMS and Obj-SO tasks. However, these same Neo-PRh animals performed as well as controls in the SOMT task that required maintenance and monitoring but used novel stimuli in

all trials. Therefore, it seemed that the Neo-PRh lesions had little effect on maintenance and monitoring working memory processes. Rather, the neonatal lesions appeared to have resulted in increased perseverative responding while performing working memory tasks with high interference. This is supported by recent findings in rodents indicating that lesions to the PRh result in impaired performance on object recognition tasks with proactive interference (Bissonette et al., 2013). Thus, we propose that the increased perseverative errors on Obj-SO in animals with Neo-PRh lesions could be due to an impaired ability to resolve proactive interference, caused by an inability to behave flexibility, i.e. to switch response strategy. To test this possibility, the present study aims to further explore the ability of Neo-PRh animals to behave flexibly.

To accomplish this goal, the same group of Neo-PRh monkeys used in Weiss et al. (2015) study will be trained on an Intradimensional/Extradimensional (ID/ED) set-shift paradigm (Dias et al., 1996). In this task, subjects learn a series of discrimination problems using compound stimuli that consist of a geometric shape with a line superimposed over it (see Figure 2). In the first stage, subjects are reinforced for responding to one of two shapes (S+). This stage measures the ability to learn stimulus-reward (S-R) associations. The second stage involves serial reversal learning. The reversal learning stages of the ID-ED task generate proactive interference and require behavioral flexibility because the same stimuli are being used while the reward contingencies are changed and must be relearned. Behavioral flexibility in this case is defined as the ability to shift responses based on changing reward contingencies in the task. The final stage is a compound discrimination (CD) stage where the same shape stimuli are used but now line stimuli are superimposed on the shapes. Subjects are rewarded for following the rule of responding to the shape (S+) and to ignore the irrelevant new stimulus dimension (line).

Therefore, the CD stage measures the ability of monkeys to maintain previously learned S-R associations while learning to ignore the new (and irrelevant) stimulus dimension. In future stages of these experiments (not included in this report), the reinforcement contingencies will again be changed such that subjects will be rewarded for responding to the previously irrelevant stimulus dimension, i.e. line instead of shape in an Extradimensional Shift Stage (EDS). By the nature of these stages, the ID-ED task is able to test both the ability to overcome interference through behavioral flexibility (Reversals) as well as the ability to shift attentional set through cognitive flexibility (EDS).

Behavioral and cognitive flexibility measured with the ID-ED task are dependent on different PFC areas including the orbitofrontal cortex (OFC) and the lateral PFC (LPFC) (Dias et al., 1996), respectively. Damage to the OFC results in impaired performance in the reversal stages, whereas damage to the LPFC results in impaired performance in the EDS stage, indicating that the OFC is important for behavioral flexibility and the LPFC is important for cognitive flexibility. These results are confirmed by findings that serial reversal learning has been associated with damage to the OFC in both human and rat models (Kazama and Bachevalier, 2012, Xue et al., 2013, Chang, 2014). In addition to the OFC, activity in other regions of the PFC such as the DLPFC and medial PFC has been associated with serial reversal learning (Xue et al., 2013, Mala et al., 2015). The PRh has direct connections to both the OFC and the LPFC, suggesting that the impaired performance of the Neo-PRh group in the study by Weiss, Nadji & Bachevalier (2015) could be due to altered connections between the PRh and the OFC, LPFC, or both. The truncated version of the ID-ED task used in this study includes tests of S-R association as well as reversal learning, which will allow us to determine whether the effects of Neo-PRh

lesions impair S-R association in the discrimination stages and/or behavioral flexibility in the reversal stages.

Hypotheses

In this task, two initial visual discrimination stages test the monkeys' ability to form stimulus-reward associations. The visual discrimination stages are followed by a series of three reversals that generate proactive interference and test the monkeys' ability to behave flexibly. The reversals are then followed by a final visual discrimination stage that consists of compound stimuli with lines superimposed over the shape stimuli that will test the monkeys' ability to selectively attend to the shapes and ignore the lines.

We hypothesize that, if the PRh is important to support S-R association learning, then monkeys with neonatal lesions of the PRh will make more errors in parts of the task that require S-R association (i.e. the discrimination stages) than controls. Additionally, if the PRh is important for behavioral flexibility, then monkeys with neonatal lesions of the PRh will make more errors in parts of the task requiring behavioral flexibility to succeed (i.e. the reversal stages).

Methods

Subjects

Nine rhesus macaques (*Macaca mulatta*) in total participated in this study. Six of these animals were given MRI-guided injections of ibotenic acid into the perirhinal cortex between 10-12 days after birth (group Neo-PRh; 3 female, 3 male) and one animal underwent the same surgical procedures, but did not receive any injections (group Neo-C; 1 female). Additionally, one animal experienced the same rearing conditions but did not undergo any surgical procedures and served

as an un-operated control (Group Neo-UC; 1 female), and one animal joined the lab as an adult and underwent sham surgery to serve as another comparison (Group Adult-C; 1 male). For this study, these three animals were combined into one control group (Group C). At the start of testing the animals were 7-16 years old.

Monkeys were fed Purina Old World Primate chow (formula 5047) and supplemented with fresh fruit enrichment. During testing, chow was restricted and the weights of the monkeys were monitored and maintained at or above 85% of the full feed weight. Water was given ad libitum. All animals were housed individually in a room with a 12 hour light/dark cycle (7AM/7PM). All subjects were born at the Yerkes National Primate Research Center breeding colony (Lawrenceville, Georgia). Neo-PRh, Neo-UC, and Neo-C animals received similar rearing and behavioral procedures including social interactions with age-matched peers and human caregivers. The Adult-C animal was mother-raised in a large colony of macaques at the Yerkes Field Station under a semi-naturalistic environment. See (Raper et al., 2013) for more details.

Previous to this study, Neo-PRh, Neo-UC, and Neo-C animals participated in cognitive testing that included tests of recognition (Zeamer et al., 2015), working memory (Weiss et al., 2015), reinforcement devaluation, and emotional regulation. The Adult-C animal participated in reinforcement devaluation, emotional regulation, and safety signal learning. All procedures were approved by the Emory Animal Care and Use Committee.

Neuroimaging

Between 10-12 days of age, subjects in the Neo-PRh group and sham operated controls underwent surgery to create excitotoxic lesions of the perirhinal cortex using ibotenic acid. MRI scans were acquired immediately before surgery to select and determine coordinates for each

injection site, as well as 6-8 days post-surgery to assess lesion extent. 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 times, 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. See Figure 2 for example FLAIR images.

Surgical Procedures

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 an appropriate plane of anesthesia. The subject's head was restrained in a stereotaxic apparatus and the subject was provided an IV drip (0.45% NaCl and dextrose) for normal hydration. Vital signs (heart and respiration rates, blood pressure, body temperature and expired CO2) were constantly monitored during the scan and 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 ridge. 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). The Neo-C group 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, 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 (25mg/kg, i.m.) once a day starting 12h prior to surgery and ending 7 days after to prevent infection.

Lesion Assessment

Histological evaluations of the animals in this study are not available because these animals will participate in future experiments. 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 used methods described in detail by Zeamer et al. (2015) and briefly here. After identifying these areas of hypersignal, corresponding regions were plotted onto matching coronal drawings 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 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. A

similar procedure was used to calculate additional damage to adjacent structures. See Table 1 for a summary of these calculations and Figure 2 for example MRI images.

Cognitive Testing

Initial training procedure

The task was delivered using an automated touch-screen apparatus. In order to acclimate the monkeys to the testing chamber and to the sound of the food dispenser, monkeys were first trained to use the touch-screen apparatus with an autoshaping program developed using the software Presentation Program. In this pretraining phase, the monkeys learned to touch clip-art images displayed on the monitor that were unrelated to the ID/ED task. When monkeys touched an image a food reward was dispensed and a sound played to indicate a correct response. Once monkeys reliably touched the stimulus appearing on the screen and ate the dispensed food rewards, the monkeys were advanced to the ID/ED task.

Intradimensional/Extradimensional set-shifting task (ID/ED)

The task consisted of three discrimination stages where one of two stimuli is rewarded. Left and right positions of the rewarded stimuli varied trial by trial in a pseudorandom fashion. The same stimulus was rewarded in each stage until performance criterion of 10 consecutive correct choices was met. A correct response was rewarded with one food pellet or M&M. The inter-trial interval for correct choices was 5s and the inter-trial interval for incorrect choices was 10s. Each subject was tested for 60 trials per day. If the learning criterion was not met, it was reset at the beginning of the next testing day.

Refer to Figure 3 for a representation of stimuli and trials. The first stage of the test was a simple discrimination (SD) stage where one of two shape stimuli was rewarded (S+). The next stage was

a series of three reversals (the simple reversals: SR1, SR2, and SR3) where the same two shape stimuli were displayed, but the previously unrewarded shape stimulus (S-) was then rewarded. This reversal of reward contingencies was repeated three times. In the reversal stages, subjects learned to inhibit responding towards a previously reinforced stimulus and switch responding to a previously non-reinforced stimulus. Subjects did not receive correction trials in the reversal stages. The final stage was a compound discrimination stage (CD) using the same shape stimuli that appear in the SDR stage, but with line stimuli (L+ and L-) superimposed on them. In the CD stage, subjects learned to respond to a specific shape regardless of what line was on it. Throughout all stages the left-right position of the rewarded stimulus was pseudorandomized, but in the CD stage the left-right position of the line stimuli varied independently of the left-right position of the shape stimuli.

Data Analysis

The experimental groups analyzed in this study include groups Neo-C (n=1), Neo-UC (n=1), C (n=1), and Neo-PRh (n=6). The three control groups (Neo-C, Neo-UC, and C) only had one monkey each, so they were combined into one group labeled Control (n=3) for analysis. The number of errors made before reaching the learning criterion of 10 consecutive correct responses (Errors to Criterion) was quantified for each animal. As an additional measure of performance, the distribution of errors was examined by separately tabulating errors that occurred earlier in learning from errors that occurred later in learning. To accomplish this, the learning criterion was adjusted to 5 correct trials in a row, and the number of errors made before reaching five in a row correct as well as the number of errors made after reaching five in a row but before moving onto the next stage were then quantified. To create an index of how these pooled errors contributed to the overall learning performance, the percentage of the total errors that occurred later in learning

(after 5) was calculated and is reported as Percent Errors After 5 (%EA5). For all planned comparisons, the degrees of freedom were adjusted when Levene's test for equality of variances was violated at $p<0.05$ according to the Satterthwaite (1946) correction.

Discrimination Stages

To determine whether there were group differences in ability to learn discrimination problems, Errors to criterion on the three discrimination stages (SD1, SD2, CD) were analyzed using a Group X Discrimination Stage ANOVA with repeated measures for the second factor. Independent t-tests were used for planned comparisons between groups at each stage. %EA5 for the three discrimination stages were also analyzed using a Group X Discrimination Stage ANOVA with repeated measures for the second factor and independent t-tests for planned comparisons between the groups at each stage.

Reversal Stages

To determine whether there were group differences in ability to learn reversal problems, Errors to criterion on the three simple reversal stages (SR1, SR2, SR3) were analyzed using a Group X Reversal Stage ANOVA with repeated measures for the second factor. Independent t-tests were used for planned comparisons between groups at each stage. %EA5 for the three reversal stages were also analyzed using a Group X Discrimination Stage ANOVA with independent t-tests for planned comparisons.

Lesion Correlation

To determine whether the extent of lesion size correlated with performance on the different stages of the task, Errors to criterion for each of the six stages was correlated with lesion size

using a Pearson Correlation. The %EA5 for each of the six stages was also correlated with lesion size using a Pearson Correlation.

Results

Discrimination Stages

The numbers of errors to reach the learning criterion for each of the discrimination stages are illustrated in Figure 4. A Group X Discrimination Stage repeated measures ANOVA revealed no significant main effect of group [$F(1,7) = 0.098$, $p = 0.763$] and stage [$F(2,14) = 1.058$, $p=0.373$], and no significant interactions between these factors [$F(2,14) = 1.859$, $p = 0.192$]. Planned comparisons between groups at each discrimination stage also indicate that Neo-PRh and Control groups did not differ on any of the discrimination stages [SD1: $t(2.144) = 0.862$, $p = 0.474$, SD2: $t(7) = -0.037$, $p = 0.972$, CD: $t(7) = -1.357$, $p = 0.217$]. These results indicate that the groups did not differ in the number of errors to reach the learning criterion at any of the discrimination stages.

The percent of total errors that occurred after the five in a row criterion (%EA5) for each of the discrimination stages are illustrated in Figure 5. A Group X Discrimination Stage repeated measures ANOVA revealed no significant main effect of group [$F(1, 7)= 0.484$, $p=0.509$], no significant effect of stage [$F(2, 14)= 1.208$, $p=0.328$] and no significant interactions between these factors [$F(2, 14)= 0.831$, $p=0.456$]. Planned comparisons between groups at each stage also indicate that Neo-PRh and Control did not significantly differ [SD1: $t(7)= 0.299$, $p= 0.774$, SD2: $t(7)= -0.316$, $p=0.761$, CD: $t(7)= -1.411$, $p=0.201$]. These results indicate that the groups made similar numbers of errors during the latter part of learning.

Reversal Stages

The numbers of errors to reach the learning criterion for each of the reversal stages are illustrated in Figure 6. A Group X Reversal Stage repeated measures ANOVA revealed no significant main effect of group [$F(1,7) = 2.200$, $p = 0.182$], a significant main effect of stage [$F(2,14) = 3.874$, $p=0.046$], and no significant interactions between these factors [$F(2,14) = 0.573$, $p = 0.576$]. Planned comparisons between groups at each reversal stage indicated that Neo-PRh and Control groups did not differ on the first two reversal stages, but the two groups did differ significantly on the third reversal stage [SR1: $t(7) = -0.731$, $p = 0.488$, SR2: $t(7) = -1.764$, $p = 0.121$, SR3: $t(7) = -2.477$, $p = 0.042$]. Pairwise comparisons were made to examine overall differences in performance between different reversal stages. These comparisons revealed that the number of errors to criterion in the third reversal stage was significantly less than the number of errors to criterion in the second reversal stage [$p=0.05$]. These results indicate that the third reversal stage was completed with fewer errors than the first and second reversals, that the Neo-PRh group made more errors overall than the Control group, and that the groups differed in the number of errors needed to complete the third reversal.

The percent of total errors that occurred after the five in a row criterion (%EA5) for each of the reversal stages are illustrated in Figure 7. A Group X Reversal Stage repeated measures ANOVA revealed a significant main effect of group [$F(1, 7)= 8.312$, $p=0.024$], no significant main effect of stage [$F(2, 14)= 0.495$, $p=0.620$] and no significant interaction between these factors [$F(2, 14)= 1.192$, $p=0.333$]. Planned Comparisons between groups at each reversal stage indicated that Neo-PRh and Control groups only differed significantly at the second reversal stage [SR1: $t(7)= -1.187$, $p=0.274$, SR2: $t(6.937)= -3.883$, $p=0.006$, SR3: $t(7)= -0.811$, $p=0.444$]. These results

indicate that the Neo-PRh group made more errors later in learning than the Control group overall, and that these differences were especially pronounced on the second reversal stage.

Lesion Correlation

The correlations between lesion extent and errors to criterion did not reach significance at any stage (SD1: r(4)= -0.804, p= 0.054, SD2: r(4)= 0.335, p=0.516, SR1: r(4)= 0.395, p= 0.438, SR2: r(4)= 0.570, p= 0.238, SR3: r(4)= 0.106, p= 0.842, CD: r(4)= -0.127, p= 0.811). The correlations between lesion extent and %EA5 also did not reach significance at any stage (SD1: r(4)= -0.600, p= 0.208, SD2: r(4)= 0.800, p= 0.056, SR1: r(4)= 0.468, p= 0.350, SR2: r(4)= 0.767, p= 0.075, SR3: r(4)= -0.401, p= 0.431, CD: r(4)= 0.724, p= 0.096).

Discussion

The findings of this study indicate that monkeys with Neo-PRh lesions were unimpaired in learning during discrimination stages, suggesting that their S-R association learning is normal. However, the same animals were impaired on the reversal stages, suggesting the presence of impairments in behavioral flexibility. These findings will be discussed in turn.

Discrimination learning stages

Data analysis indicated that the Neo-PRh group performed similarly to the Control group on the three discrimination stages. These data suggest that the PRh is not necessary to support S-R association learning. The measure of percent of total errors that occurred after the 5 in a row criterion (%EA5) also indicated that the distribution of errors in each stage did not differ between the two groups. The present results corroborate earlier findings from monkeys with similar PRh lesions created in adulthood. In this previous study (Hampton and Murray, 2002), monkeys with adult-onset PRh lesions performed as well as controls when learning visual

discrimination problems. Taken together, these findings demonstrate that the PRh is not necessary to support simple S-R association learning.

Reversal learning stages

Performance differed across the reversal stages. In both groups performance improved across the reversals. Specifically, the third reversal was completed with fewer errors than the second reversal. Serial reversal learning involves a certain amount of "learning to learn" in that learning the reward contingencies of later reversals can be facilitated by knowledge of previous reversals. Thus, the findings indicate that damage to the PRh does not alter this ability. Although Neo-PRh monkeys made similar number of errors as Control on the first two reversal stages, planned comparisons revealed that they made significantly more errors than controls on the last reversal stage. This impairment corroborates a similar reversal learning impairment reported in rhesus macaques with adult-onset PRh lesions (Hampton and Murray, 2002). Such an impairment could be explained by an inability of the Neo-PRh monkeys to rapidly switch their responses when a new reversal occurred due to the interference caused by the reward contingencies learned in previous reversals.

To further investigate this possible impairment was to analyze the distribution of the errors across a reversal, in that animals that were more susceptible to interference may make more errors later in the reversal than earlier. To further investigate this difference in performance, the percentage of the total errors that occurred later in learning (after 5 correct trials) was calculated and is reported as Percent Errors After 5 (%EA5). Analysis revealed a main effect of group, but no main effect of stage and no interaction between the two factors for the %EA5 measures. The significant main effect of group indicated that Neo-PRh monkeys made a larger proportion of their errors in the later trials of a reversal. This finding suggests that the Neo-PRh monkeys in

this study were impaired on the reversal stages because of an inability to sustain behavioral flexibly long enough and overcome proactive interference from the previous encounters with the objects.

This study explored the proposal that impaired S-R association learning and impaired behavioral flexibility as two possible explanations for the Neo-PRh monkeys' significantly worse working memory performance than controls in the Obj-SO task (Weiss et al., 2015). The results indicate that a lack of behavioral inhibition rather than impaired S-R learning could be at the source of increased perseverative errors in the working memory task. While the results of this study indicate that there is some impairment in behavioral flexibility in Neo-PRh monkeys, a second possibility is that the Neo-PRh monkeys could have impairments with cognitive flexibility. Here, cognitive flexibility is defined as the ability to flexibly switch cognitive strategy and can be tested in the Extradimensional Shift (EDS) stage of the ID/ED task. In the EDS stage, monkeys must learn to ignore the previously rewarded dimension of shape and, instead, attend to the previously ignored line stimuli through use of cognitive flexibility. Future work will address the possibility that the neonatal PRh lesions impacted cognitive flexibility by testing the same monkeys on the EDS stages of the ID/ED task. If the results of this future work indicate that Neo-PRh monkeys are impaired at the EDS stage, then it will suggest that Neo-PRh lesions impair both behavioral and cognitive flexibility. However, if the results indicate that Neo-PRh monkeys are not impaired at the EDS stage, then it will suggest that Neo-PRh lesions impair behavioral flexibility but spare cognitive flexibility.

Relevance to Neurological Disorders

The ID/ED task has been used clinically to characterize flexible behavior and cognition in neurological disorders such as Parkinson's disease, autism, and schizophrenia. A common symptom of these disorders is impaired executive function, which is tested in the ED shift stage of the ID/ED task through attentional set shifting (i.e. shifting attention from one set of stimuli to another). Patients with Parkinson's disease are impaired in the ED stage of the ID/ED task, suggesting that cognitive flexibility is impaired in the onset of the disease and confirming the impairments to executive function in Parkinson's disease (Gauntlett-Gilbert et al., 1999). One of the primary symptoms of Autism Spectrum Disorders (ASD) is behavioral rigidity in the form of stereotyped movements or restricted interests. This symptom is directly related to behavioral flexibility and can be tested using the ID/ED task. Patients with ASD are impaired in the ED reversal stages of the ID/ED task but not the other reversal stages or the ED shift stage (Yerys et al., 2009). Schizophrenia is characterized by impaired attentional set-shifting which has been tested using the ID/ED task. Patients with schizophrenia are impaired in differing stages of the ID/ED task depending on symptomatology, but overall require more trials to complete the task than healthy controls (Ceaser et al., 2008, Barnett et al., 2010). Patients with these neurological diseases demonstrate impairments in the ID/ED task that could increase understanding of the mechanisms behind their symptoms. Studying the basic science behind the ID/ED task could additionally increase our understanding of the impairments in executive function in these neurological diseases. For this reason, our study has the potential to further knowledge of both typically developing executive function and the impairments present in patients with neurological diseases.

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Table 1. Percent of Intended and Unintended Damage. Previously reported by (Zeamer et al., 2015). Displayed in this table are the percent damage to the perirhinal cortex and adjacent structures for the six subjects in Group Neo-PRh estimated from pre- and post-surgery coronal FLAIR images. These are listed as follows: L%: the percent damage to the left hemisphere, R%: the percent damage to the right hemisphere, X%: average damage to both hemispheres, W%: weighted average damage to both hemispheres, an index that takes into account lesion asymmetry (W%=(L%xR%)/100). Adjacent areas also displayed in this table include the entorhinal cortex (ERh), amygdala (AMY), hippocampal formation (HF), visual cortex (TE), and cytoarchitectonic fields of the parahippocampal gyrus (TH/TF).

Subjects	**PRh**				**ERh**				**TE**			
	L%	R%	X%	W%	L%	R%	X%	W%	L%	R%	X%	W%
Neo-PRh-1	89.76	76.91	83.34	69.04	28.51	2.28	15.39	0.61	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	3.66	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	34.23	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
Avg.	*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	3.47	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	3.22	0.32	1.77	0.01
Avg.	*1.17*	*0.66*	*0.91*	*0.05*	*2.00*	*2.96*	*2.48*	*0.18*	*1.12*	*0.05*	*0.81*	*0.00*

Figure 1. Direct connections from the Perirhinal Cortex and indirect connections from the Hippocampus to the Prefrontal Cortex.

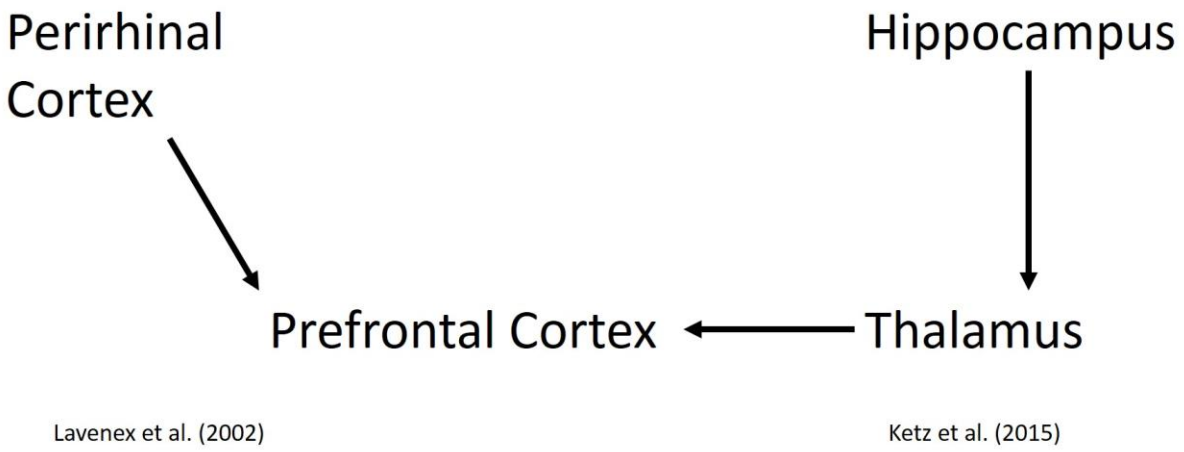

Figure 2. Example coronal pre-surgical T1 MR images (left column) and post-surgical FLAIR MR (right column) images from case Neo-PRh-3. The pre-surgical T1 MR images are in the left column and the post-surgical FLAIR MR images are in the right column. The edema caused by cell damage from the injection of ibotenic acid can be seen in the FLAIR images as white areas. Images courtesy of (Zeamer et al., 2015). Red arrows indicate the rhinal sulcus while yellow circles indicate the regions of hypersignal.

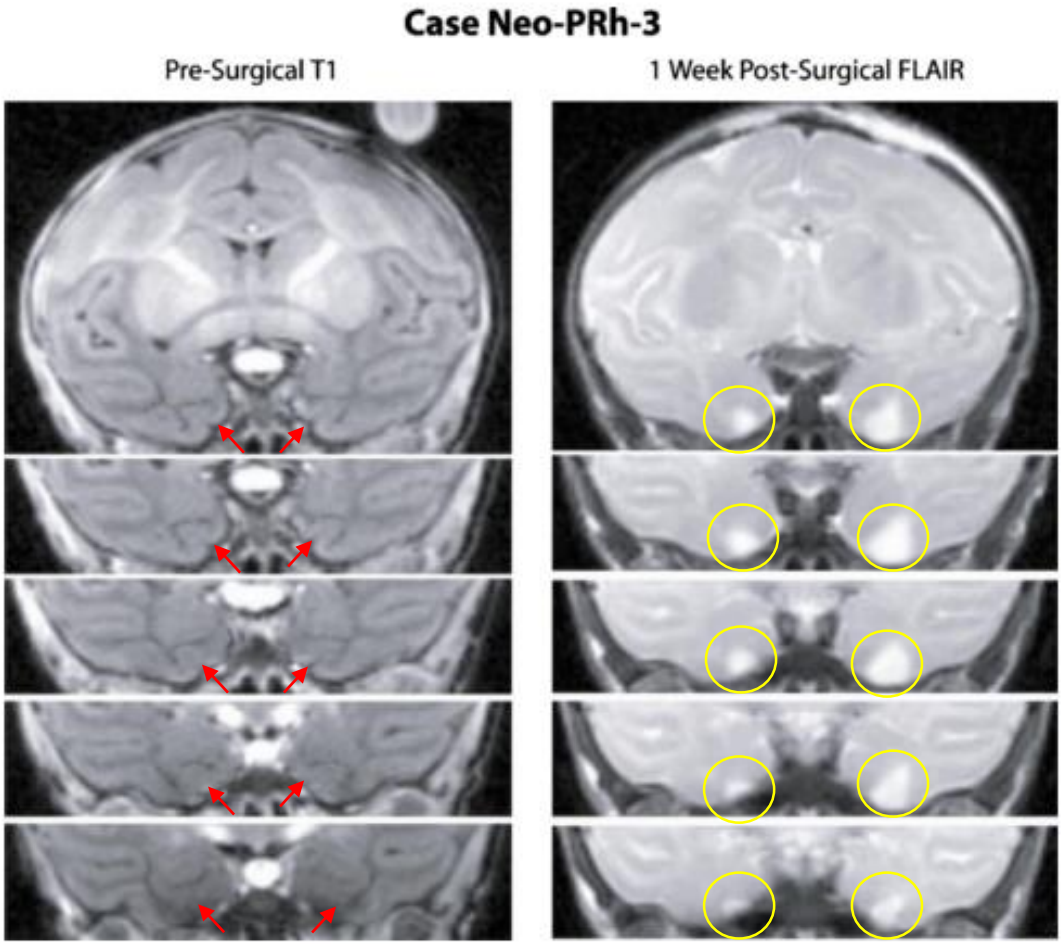

Figure 3. Stages of the truncated ID/ED task. The truncated ID/ED task consists of six stages with three visual shifts and one set of three serial reversals in between. The S+ labeled stimuli are rewarded shapes while the S- stimuli are non-rewarded shapes.

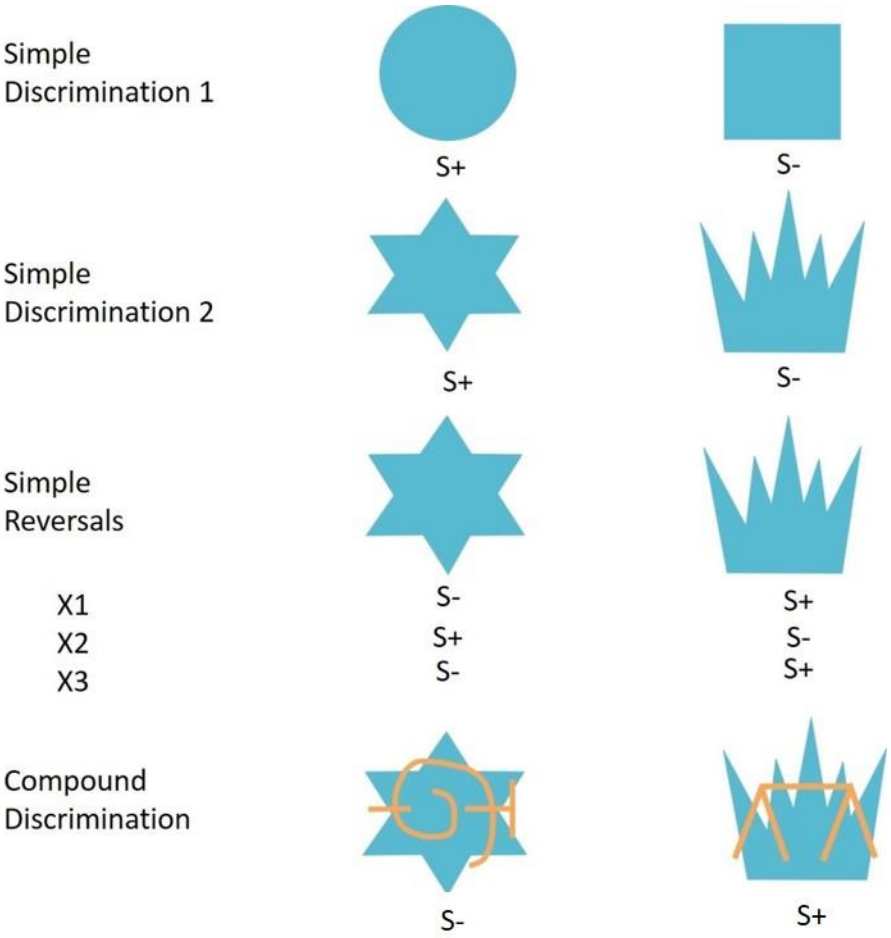

Figure 4. The number of errors to criterion of group Neo-PRh (displayed in grey) compared to group Control (displayed in white) across the first and second discrimination stages and the final compound discrimination stage. Bars represent +/- 1 standard error.

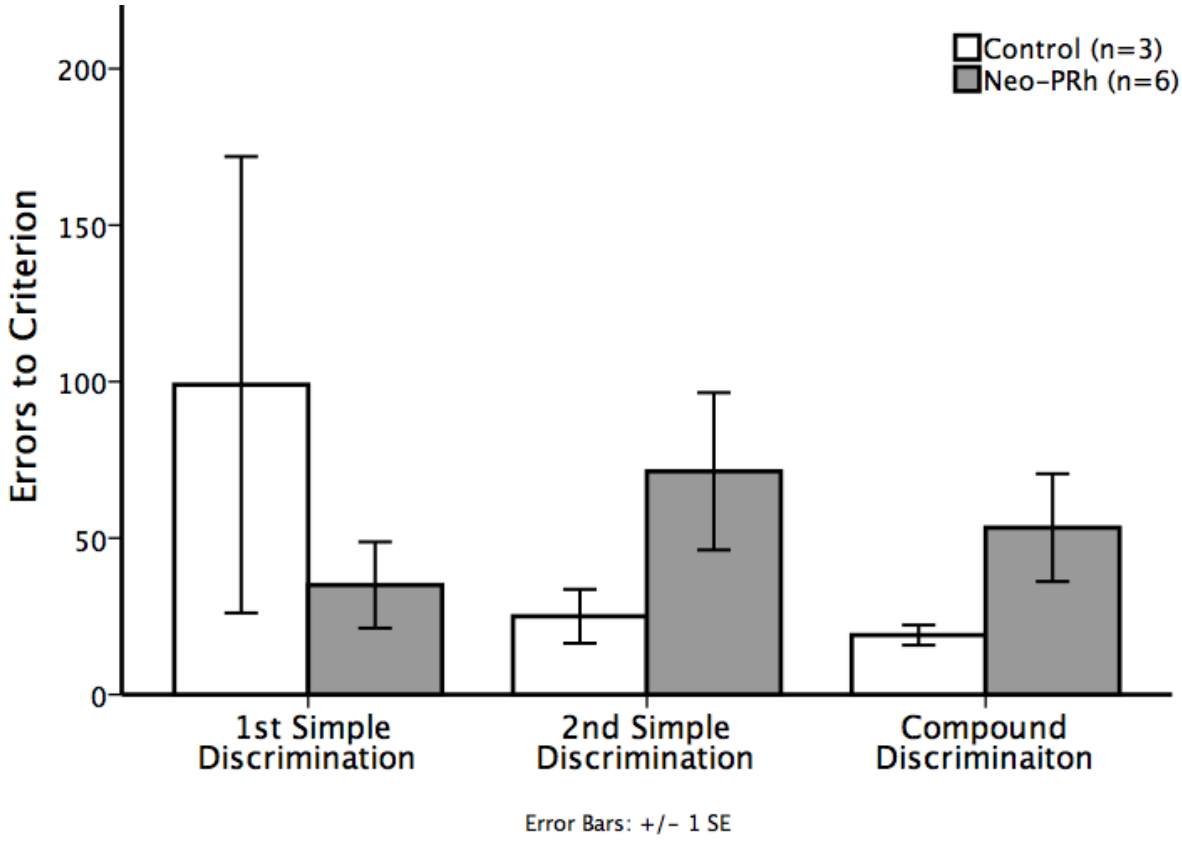

Figure 5. The percent of total errors that occurred after the 5 in a row criterion of group Neo-PRh (displayed in grey) compared to Control (displayed in white) across the first and second discrimination stages and the final compound discrimination stage. Bars represent a standard error of 1.

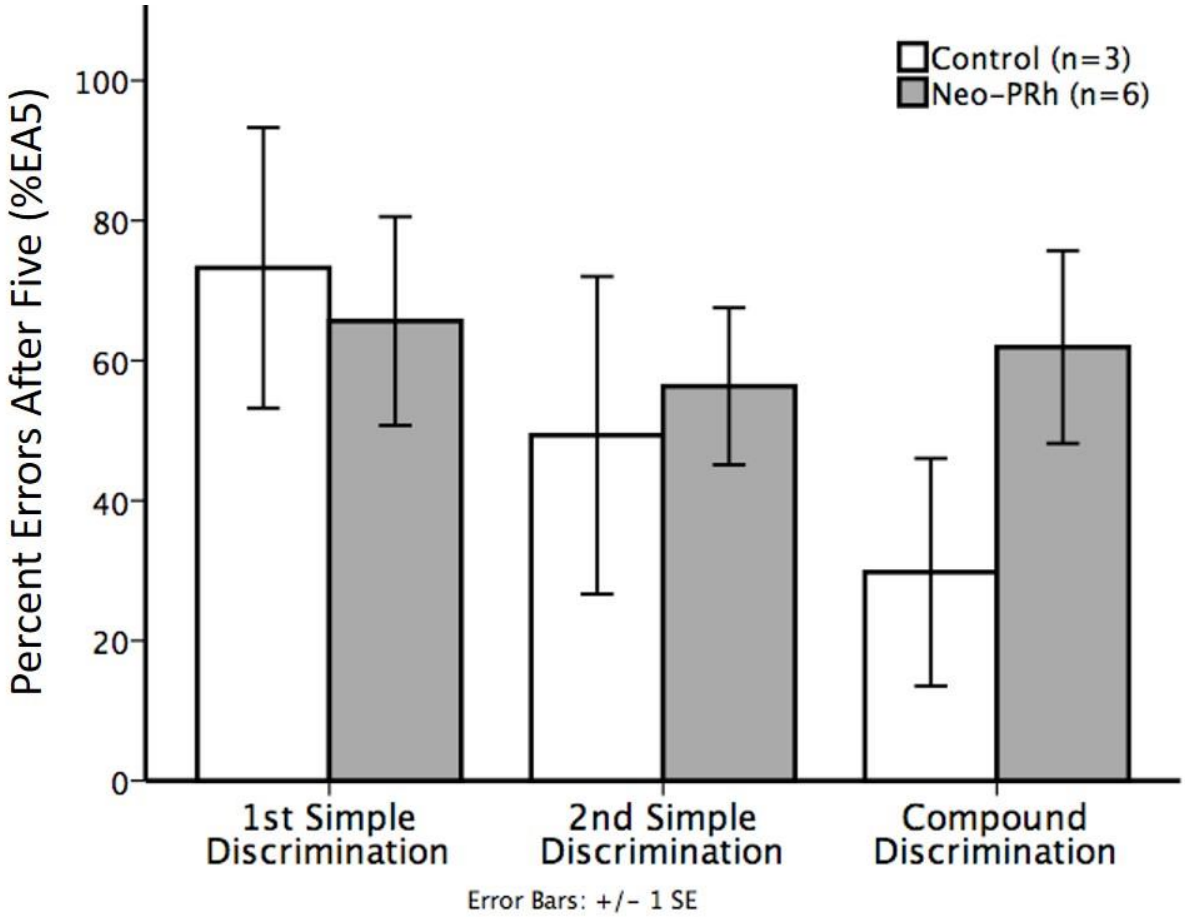

Figure 6. The number of errors to criterion of group Neo-PRh (displayed in grey) compared to group Control (displayed in white) across the three simple reversal stages. Bars represent a standard error of 1.

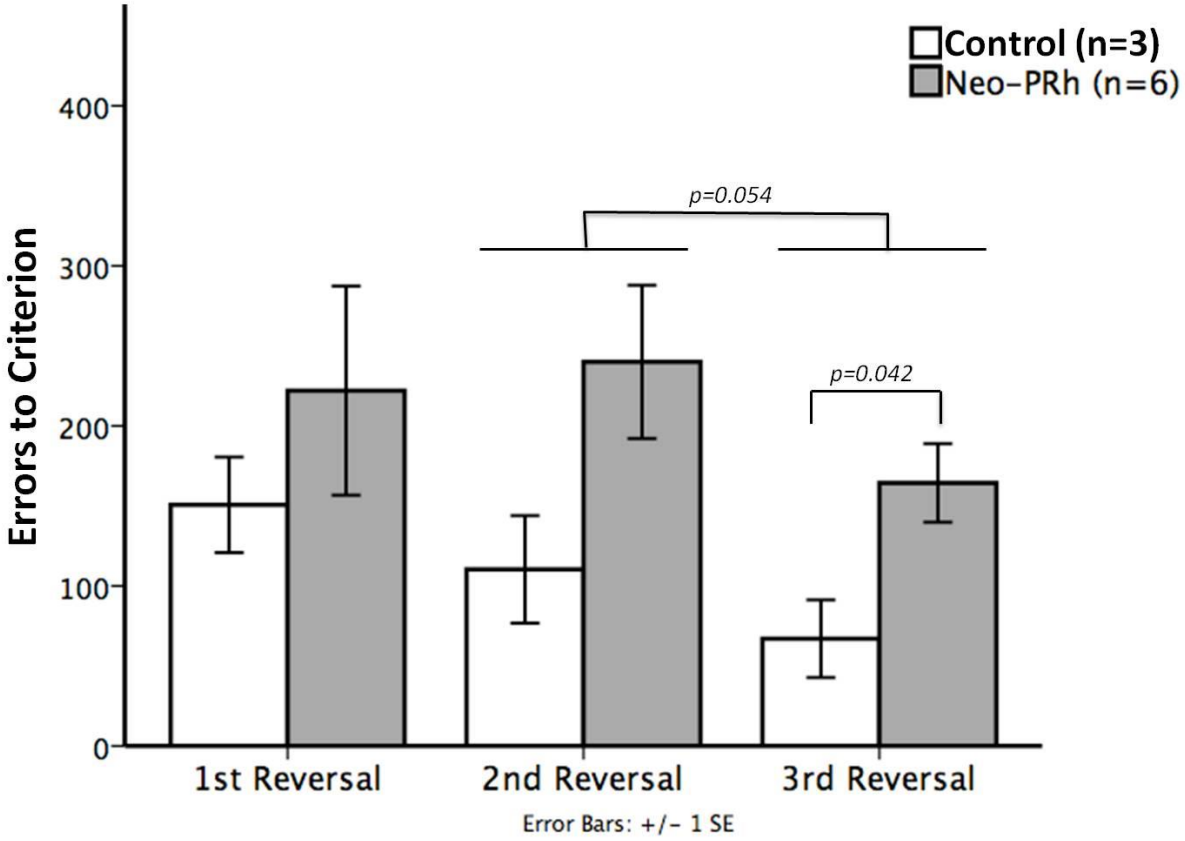

Figure 7. The percent of total errors that occurred after the 5 in a row learning criterion of group Neo-PRh (displayed in grey) compared to group Control (displayed in white) across the three reversal stages. Bars represent a standard error of 1.