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Muscimol, AP5, or scopolamine infused into perirhinal cortex impairs two-choice visual discrimination learning in rats

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ABSTRACT

The perirhinal cortex (PRh) has been strongly implicated in object recognition memory and visual stimulus representation. Studies of object recognition have revealed evidence for the involvement of several neurotransmitter subsystems, including those involving NMDA (*N*-methyl-D-aspartic acid) and muscarinic cholinergic receptors. In the present study, we assessed the possible involvement of PRh and related receptor subsystems in two-choice visual discrimination learning by Lister Hooded rats tested in touchscreen-equipped operant boxes. In Experiment 1, daily pre-training inactivation of PRh with the $GABA_A$ receptor agonist muscimol (0.5 μg/hemisphere) significantly impaired acquisition of the two-choice visual discrimination. In Experiment 2, daily pre-training blockade of either NMDA or muscarinic receptors in PRh with AP5 (5.9 μg/hemisphere) or scopolamine (10 μg/hemisphere), respectively, impaired task acquisition. These results parallel the findings from object recognition studies and suggest a generality of neurotransmitter receptor involvement underlying the role of PRh in both object recognition memory and visual discrimination learning. The involvement of PRh in both types of tasks may be related to its role in complex visual stimulus representation.

1. Introduction

Research has indicated that the perirhinal cortex (PRh) of the medial temporal lobe is essential for object recognition memory, particularly when object information must be retained across a delay interval (Buffalo, Reber, & Squire, 1998; Meunier, Bachevalier, Mishkin, & Murray, 1993; Winters, Forwood, Cowell, Saksida, & Bussey, 2004). Indeed, PRh appears to mediate memory acquisition, consolidation, and retrieval in the spontaneous object recognition (SOR) task for rats (Winters & Bussey, 2005c), and these functions have recently been shown to depend differentially on various neurotransmitter receptors in PRh (Barker, Bashir, Brown, & Warburton, 2006; Winters & Bussey, 2005a; Winters, Saksida, & Bussey, 2006).

The involvement of PRh in object recognition memory may be related to its broader role in object identification and the representation of complex visual stimulus information. Recent work has implicated PRh in visual discrimination and complex perceptual functions, and these findings have supported suggestions that PRh is involved in both perception and memory by virtue of its anatomical connectivity with cortical areas in the ventral visual processing stream (Buckley & Gaffan, 1998; Bussey & Saksida, 2002; Murray & Bussey, 1999; Murray, Bussey, & Saksida, 2007). In light of the putative perceptual-mnemonic functions of PRh, it is reasonable to suggest that this cortical region could be involved in learning and memory tasks other than object recognition that require complex visual stimulus information processing. Indeed, findings from permanent lesion studies in rats and monkeys indicate that PRh is important for the learning and performance of visual discrimination tasks, providing the perceptual requirements of these tasks are sufficiently high (Bussey, Saksida, & Murray, 2002, 2003; Eacott, Machin, & Gaffan, 2001). The learning and performance requirements of visual discrimination tasks differ substantially from the SOR task, and thus a comprehensive analysis of PRh involvement in these paradigms is necessary to determine the specific contributions of PRh to different learning and memory tasks.

Previous results implicating PRh in visual discrimination processes come primarily from lesion studies, which, although highly valuable, tell us little about the specific neural mechanisms underlying the role of PRh in learning and memory. In the present study, we asked whether the same types of mechanisms demonstrated to operate within PRh during object recognition memory could be shown for another, very different type of learning task. To this end, we developed a rat version of the touchscreen-based visual discrimination tasks used for monkeys. Rats were trained on a two-choice visual discrimination with computerized photographic stimuli presented in an operant touchscreen apparatus. The use of

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the computerized touchscreens allows a great deal of control over the nature of the visual stimuli being presented. Furthermore, use of rats as subjects facilitates the performance of high throughput studies in which very specific neurobiological manipulations can be conducted to analyze the neural bases of visual discrimination learning. Accordingly, in the present study, we assessed the effects of transient receptor blockade in PRh on task acquisition. In Experiment 1, the involvement of PRh in the acquisition of the two-choice visual discrimination was assessed by giving rats bilateral intra-PRh infusions of the $GABA_A$ receptor agonist muscimol before the start of daily training sessions. In Experiment 2, the receptor mechanisms underlying PRh involvement in acquisition of the task were assessed. Recent research has implicated NMDA glutamate receptors and muscarinic cholinergic receptors in PRh in the acquisition of object information in the SOR task (Barker, Warburton, et al., 2006; Warburton et al., 2003; Winters & Bussey, 2005a; Winters et al., 2006). We therefore trained rats on the two-choice visual discrimination task with bilateral intra-PRh infusions of the NMDA receptor antagonist AP5 or the muscarinic receptor antagonist scopolamine before the start of daily training sessions. Each of the three drugs disrupted two-choice visual discrimination learning. These results mirror the findings from object recognition studies and indicate a generality of mechanisms underlying the role of PRh in object recognition memory and two-choice visual discrimination learning.

2. Methods and materials

2.1. Subjects

The subjects were 30 adult male Lister Hooded rats (Harlan, Olac, Bicester, UK), weighing 270–320 g prior to the start of behavioral training and housed in pairs in a room with a 12-h light: 12-h dark cycle (lights on at 7:00 P.M.). Different batches of rats were used for each experiment. The number of rats used in each experiment was as follows: Experiment 1, 13 rats; Experiment 2, 17 rats. All behavioral testing was conducted during the dark phase of the cycle. During testing, rats were fed approximately 15 g of laboratory chow following daily behavioral sessions to maintain weights at 85–90% of free-feeding body weight. Water was available ad libitum throughout the experiment. All experimentation was conducted in accordance with the UK Animals (Scientific Procedures) Act, 1986.

2.2. Apparatus

Preliminary training and behavioral testing were carried out in eight automated touchscreen testing chambers. The apparatus consisted of a standard modular testing chamber housed within a sound-attenuating box (Med Associates Inc., Vermont, USA). The box was fitted with a 28 volt DC fan for ventilation and masking of extraneous noise. The inner operant chamber ($30.5 \times 24.1 \times 8.25$ cm; Med Associates Inc., Vermont, USA) consisted of a metal frame, clear Perspex walls and a stainless steel grid floor. A pellet receptacle (magazine) attached to a 45 mg pellet dispenser was situated outside of the box. A 3 W houselight and tone generator (Med Associates Inc., Vermont, USA) were fitted to the back wall of the chamber. The magazine was illuminated by a 3 W light bulb and fitted with photocell head entry detectors to detect the rats' presence in that area of the testing chamber.

At the end of the box opposite the magazine was a flat screen monitor equipped with an infrared touchscreen (Craft Data Ltd., Bucks, UK; ELO Touchsystems, Wiltshire, UK; Displaze, Aylesbury, UK) mediated by ELO touchscreen software (ELO Touchsystems Inc.). The use of a touchscreen that uses infrared photocells means that the rat is not required to exert any pressure on the monitor screen in order for a nose-poke to be detected; a nose-poke is registered during the rat's natural sniffing behavior toward stimuli presented on the screen. A Perspex 'mask' was located in front of the touchscreen, rising up 15 cm from the grid floor of the operant chamber to support a 'shelf' extending 7 cm from the surface of the mask supported by springs (to prevent the rat climbing onto it). The effect of the shelf was to force the rat to stop, rear up and stretch toward the stimuli with a head-on approach, thus facilitating the rats' attention to the stimuli (Fig. 1A). Computerized visual stimuli were displayed in the area of the touchscreen immediately above the mask. This region of the touchscreen was divided into two halves by a 10 cm metal rod affixed to the back of the mask in the space between the touchscreen and mask; the divider created two discrete response areas on the touchscreen in which stimuli could be presented.

2.3. Touchscreen pre-training

Rats were initially shaped to collect food pellets from the food magazine. During the first session, rats were habituated to the testing chamber. Pellets were placed in the magazine and the rats left in the testing chamber for 15 min. In the next session, the rats were trained to collect pellets that were delivered every 20 s together with the illumination of the magazine light and presentation of the tone. During this stage, training stimuli (40 stimuli varying in brightness, shape, and pattern) were presented on the touchscreen, one per trial in either of the two response areas for 20 s. Multiple training stimuli were used to minimize the development of biases to particular features of stimuli. A single pellet was delivered immediately following stimulus offset. If the rat touched the stimulus, however, the stimulus disappeared and the rat was rewarded with a pellet. Completion of this stage, however, did not depend on the rat touching the stimuli on the screen, and rats were removed from the testing chamber after 30 min regardless of the number of trials completed.

In the next session rats were required to respond at the touchscreen in order to gain reward. On each trial, a training stimulus was shown in one of the two response windows. The stimulus remained on the screen until the rat responded to it, after which the rat was rewarded with a pellet, tone and illumination of the magazine light. This was followed by a 20-s inter-trial interval (ITI), after which the stimulus for the next trial was displayed on the screen. Once rats were successfully completing 50 trials in a 30-min session, they were required to initiate each trial. After a choice had been made, the first head entry into the magazine following the ITI resulted in the stimulus being displayed for the next trial. This meant that on every trial the rat was situated at the back of the testing chamber when the stimulus was displayed. The first head entry into the magazine during a session resulted in the stimulus being displayed for the first trial.

Once the rat was able to obtain 50 pellets within 30 min, it was moved onto the next stage, in which punishment for incorrect responses and a correction procedure were introduced. The task was now effectively a two-choice discrimination, run in the same way as in the task proper (see below), but this pre-training version simply required a choice between the response area containing a stimulus, and the one containing no stimulus. On a given trial a stimulus was presented on the computer screen in one of the two response windows. The rat was required to approach the touchscreen and make a response via a nose-poke. Correct responses were followed by the disappearance of the stimulus and the presentation of a pellet and tone concomitant with the illumination of the food magazine, followed by a 20 s ITI. Incorrect responses resulted in the disappearance of the stimuli and the houselight being extinguished for a time-out period of 5 s,

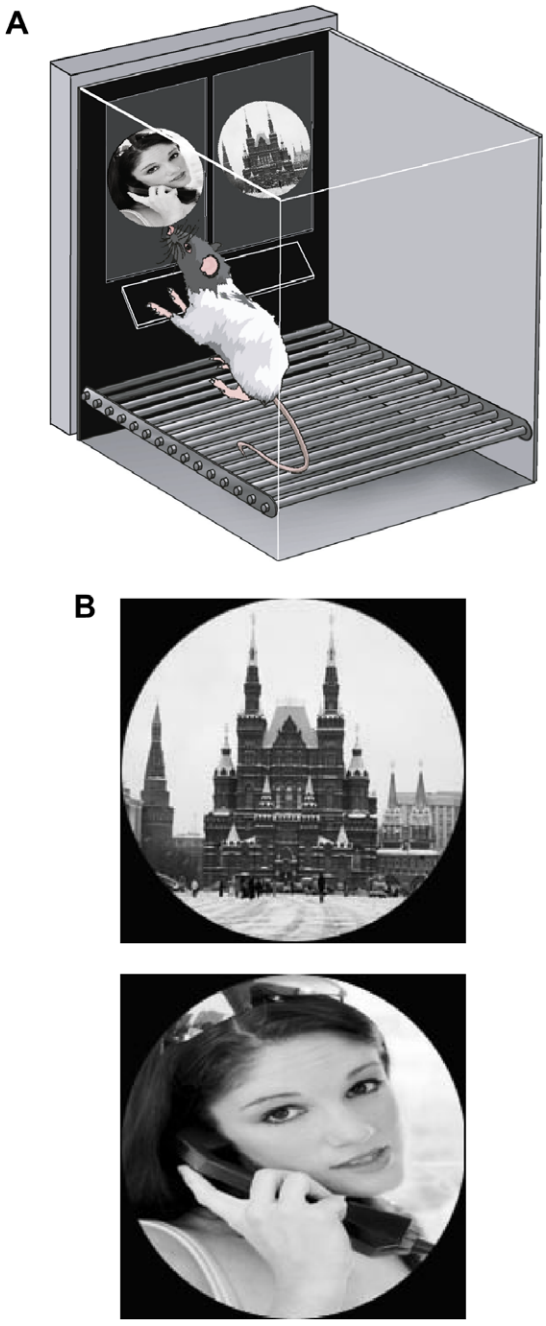


Fig. 1. A. Diagram of a rat responding at the touchscreen monitor. In the final version of the two-choice visual discrimination task, the S+ and S− were presented together on each trial. Rats were required to rear up and nose-poke the correct stimulus (S+) for a pellet reward. B. The photographic stimuli used for the two-choice visual discrimination task. Stimuli were grey scale and are shown actual size (5.27 cm diameter). The stimuli were presented on the computer-controlled touchscreens with a black background and therefore appeared as circular photographic images when presented in training sessions. In each experiment each stimulus was used approximately equally as the rewarded S+ or the nonrewarded S−.

followed by the ITI. A correction procedure was implemented whereby the trial was repeated until the rat made a correct choice. Rats were run on this final stage of pre-training until they managed to perform 60 trials in less than 1 h with 90% correct for two consecutive days. Rats then underwent surgery for guide cannula implantation (see below). After surgery, they were allowed to recover for 7–10 days before resumption of behavioral training. The initial two sessions following recovery consisted of 'reminder' sessions to confirm that all rats were still capable of performing the basic aspects of touchscreen responding. These two sessions were run in exactly the same way as the final pre-training sessions before surgery. Training of the two-choice visual discrimination task then commenced.

2.4. Two-choice visual discrimination

Once the rats had completed pre-training, they were then trained on a two-choice visual discrimination with daily pre-session bilateral infusions of drug or saline into PRh as described below. Each session consisted of a maximum of 60 trials or the number of trials completed in 1 h. The ITI was 20 s, and rats were required to initiate each trial by responding at the magazine. Following trial initiation, a pair of stimuli would appear on the screen, one in each of the two response areas; one stimulus was the correct S+ and the other the incorrect S− (Fig. 1A). A nose-poke to the S + resulted in a tone, magazine light, and a reward pellet. Incorrect responses resulted in a 5 s time-out period followed by the correction procedure. The S+ and S− were brightness-matched computerized photographic stimuli circular in shape with a 5.27 cm diameter (Fig. 1B). Both discriminative stimuli were presented an equal number of times during a session. The left–right arrangement of the stimuli was determined pseudorandomly across trials, with a constraint that a given stimulus could not appear on the same side of the screen on more than three consecutive trials. The same pair of discriminative stimuli was used for Experiments 1 and 2, and the specific stimuli are shown in Fig. 1B. These stimuli were novel to the animals at the start of two-choice visual discrimination training and did not resemble any of the stimuli used in the pre-training stages. Each experiment was counterbalanced such that half of the rats in each group received one stimulus in the pair as the S+, and the other half received the other stimulus as the S+. In both experiments, rats were run for 10 visual discrimination training sessions.

2.5. Surgery

For each experiment, all rats were implanted bilaterally with 22-gauge indwelling guide cannulas after reaching the final criterion for touchscreen pre-training. Prior to surgery, all animals were deeply anaesthetized by intraperitoneal (i.p.) injection (60 mg/kg) of sodium pentobarbital (Sagatal; Rhône Mérieux, Essex, UK) and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) with the incisor bar set at −3.2 mm. The scalp was cut and retracted to expose the skull. Holes were drilled and the guide cannulas implanted according to the following coordinates, measured relative to the skull at bregma (Paxinos & Watson, 1998): anteroposterior −5.5 mm, mediolateral ±6.6 mm, dorsoventral −6.5 mm. The ends of the guide cannulas were, thus, located just dorsal to the main target area. The cannulas were secured to the skull using four jeweler screws and dental acrylic. Obturators cut to extend 1.1 mm beyond the tip of the guide cannulas and with an outer diameter of 0.36 mm were inserted into the guides and remained there except during infusions. At the completion of each surgery, the skin was sutured, and an antibiotic powder (Acramide; Dales Pharmaceuticals, Skipton, UK) was applied. Animals were allowed to recover for at least seven days prior to the beginning of behavioral testing.

2.6. Infusion procedure

In Experiment 1, two groups of rats received bilateral infusions of either physiological saline (0.9% sodium chloride, pH 7.0; Aquapharm, Animalcare Limited, York, UK; $n = 6$) or muscimol hydrobromide (1 mg/ml in physiological saline; Sigma, Poole, UK; $n = 7$) 15 min before the start of each of 10 daily two-choice visual

discrimination training sessions. In Experiment 2, three groups of rats received bilateral infusions of physiological saline ($n = 4$), AP5 [D(-)-2-amino-5-phosphonopentanoic acid; 5.9 mg/ml in physiological saline; Sigma, Poole, UK; $n = 6$], or scopolamine hydrobromide trihydrate (10 mg/ml in physiological saline; Sigma, Poole, UK; $n = 7$), 15 min before daily training sessions. All infusions took place in a preparation room separate from the behavioral testing area. Animals were gently restrained by the experimenter throughout the infusion process. The obturators were removed, and 28-gauge infusion cannulas, which were cut to extend 1 mm beyond the tip of the guides, were inserted into the guides. Bilateral infusions were conducted simultaneously using two 1-μl Hamilton syringes, which were connected to the infusion cannulas by propylene tubing. The syringes were driven by a Harvard Apparatus precision syringe pump, which delivered infusate to each hemisphere at a rate of 0.5 μl/min. In Experiment 1, 0.5 μl of muscimol or saline was infused over 1 min into each hemisphere before daily training sessions. In Experiment 2, drugs or saline were infused in a volume of 1 μl per hemisphere over 2 min before daily sessions. The infusion cannulas were left in place for an addition 1.5 min to allow for diffusion of the infusate. The infusion cannulas were then removed, and the obturators replaced. For the 15 min period between infusion and the start of the training session, the rats were placed back into their cages in the holding room adjacent to the testing room. In both experiments, in each of the two 'reminder' sessions prior to the beginning of two-choice visual discrimination training, rats experienced a 'mock' infusion identical in all aspects to the procedure described above, except that the injection cannulas contained no liquid. This was done to habituate the animals to the infusion procedure.

2.7. Histology

Following behavioral training, rats were anaesthetized by i.p. injection of 2 ml of Dolethal (Rhône Mérieux) and perfused transcardially with 100 ml of phosphate buffered saline (PBS, pH 7.4), followed by 250 ml of 4% paraformaldehyde (PFA, pH 7.4). The brains were removed, postfixed in 4% PFA at 4 °C for 24 h and then immersed in 25% sucrose in PBS until they sank. Coronal sections (60 μm) were cut on a freezing microtome through the extent of PRh, and every fifth section was mounted on a gelatin-coated glass slide and stained with cresyl violet. Slides were examined under a light microscope to verify the cannula placements.

2.8. Fluorescent muscimol spread analysis

The distribution of fluorophore-conjugated muscimol (FCM; 1 mg/ml in physiological saline; Molecular Probes) was assessed in eight rats not run during the behavioral sessions (Allen et al., 2008). These rats underwent the same surgical procedures as those in the behavioral experiments and were habituated with two mock infusion days prior to the actual infusion of FCM. Infusions were conducted in the manner described above for the behavioral experimental sessions. Four rats were infused bilaterally into PRh with 1 μl of FCM delivered over 2 min, while the other four rats received 0.5 μl over 1 min. Half of the rats in each group were then euthanized and perfused as described above 15 min following the intracranial infusion of FCM. The other four rats were euthanized and perfused 1 h and 15 min following intracranial FCM infusions. These conditions were done to assess the spread of infusate expected to result from infusions in the volumes used for both behavioral experiments (i.e., 0.5 and 1 μl), as well as to compare the relative spread observed at time points corresponding to two relevant points in behavioral testing – the beginning of the behavioral session (i.e., 15 min after drug infusion) and the end of the session (i.e., 1 h and 15 min after infusions).

The brains were removed, postfixed in 4% PFA at 4 °C for 24 h and then immersed in 25% sucrose in PBS until they sank. Coronal sections (60 μm) were cut on a freezing microtome through the extent of PRh, and every fifth section was mounted on a gelatin-coated glass slide for qualitative spread analysis. Slides were examined under a confocal microscope to verify the regions of infusate spread under the different infusion and perfusion timing conditions. For each brain, a parallel series of 60-μm sections was stained with cresyl violet to verify the cannula tip locations.

2.9. Data analysis

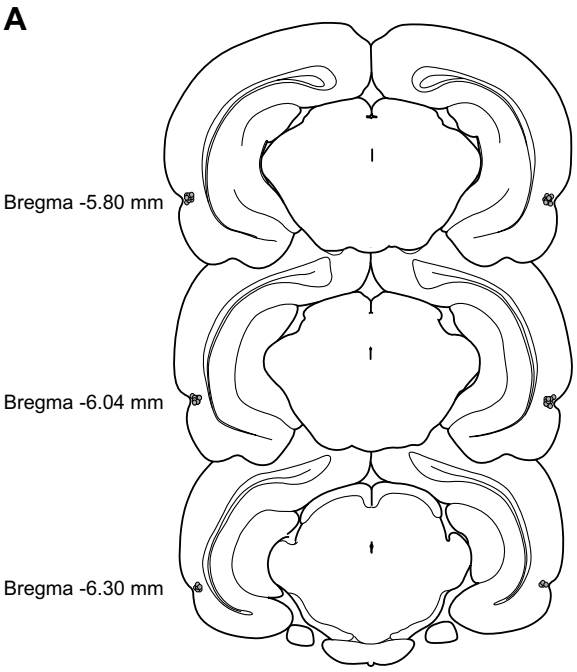
Task performance was measured as the average daily percent correct for each group. The daily percent correct was analyzed for each experiment by a two-way (drug by session) repeated measures analysis of variance (ANOVA). Three control measures were also analyzed in each experiment: (1) The number of trials completed on average across the 10 training sessions; (2) The average response latency, i.e., the time required on average for a rat to respond at the touchscreen after the appearance of the stimuli; and (3) The average magazine latency, i.e., the time required for a rat to collect the reward pellet from the magazine after a correct response. The means gathered from across the 10 training sessions were analyzed in separate independent-samples t-tests for each of the three control measures for Experiment 1. For Experiment 2, the 10-session means for each group were analyzed in separate univariate ANOVAs for each of the three control measures. Student Newman–Keuls was used to analyze between-subjects effects. All statistical analyses were conducted with a significance level of $\alpha = 0.05$.

3. Results

3.1. Cannula placements and infusate spread analysis

Rats in all experiments had cannulas implanted bilaterally within PRh to allow delivery of drugs. All rats included in the behavioral analyses had guide cannulas located bilaterally with injection needle tips terminating in PRh near the border between areas 35 and 36 within cortical layers 2–5 (Burwell, 2001). These placements were consistently located between 5.80 and 6.30 mm posterior to bregma (Fig. 2A), the approximate mid-section of the rostral–caudal extent of PRh.

Data from our own laboratory using similar infusion parameters to those employed in the current study, as well as other published assessments suggest that the spread of infusate should have been limited to an area of tissue approximately 0.5–1 mm in radius from the tip of the infusion cannula (Martin, 1991; Winters & Bussey, 2005c). This would include the majority of PRh tissue, with only minor penetration into adjacent areas such as entorhinal cortex or area TE. Our analysis of the spread of FCM supports these assumptions (Fig. 2B). All rats infused with FCM for spread analysis had cannula tip placements similar to those from the behavioral experiments. Qualitative analysis of the diffusion area of FCM in the brains of these rats indicated a relatively discrete area of spread that included almost the entire rostral–caudal extent of PRh. Fig. 2B illustrates the typical diffusion area within PRh in a representative section. In general, the spread of FCM was found to be even more localized than that previously reported for radiolabeled lidocaine infused in a similar manner into PRh (Winters & Bussey, 2005c), and there were no substantial differences between the spread observed in animals perfused 15 min or 1 h and 15 min post-infusion. Moreover, although the 1-μl infusions spread further than the 0.5-μl infusions, this difference was minor, and both conditions resulted in diffusion primarily limited to PRh tissue. In



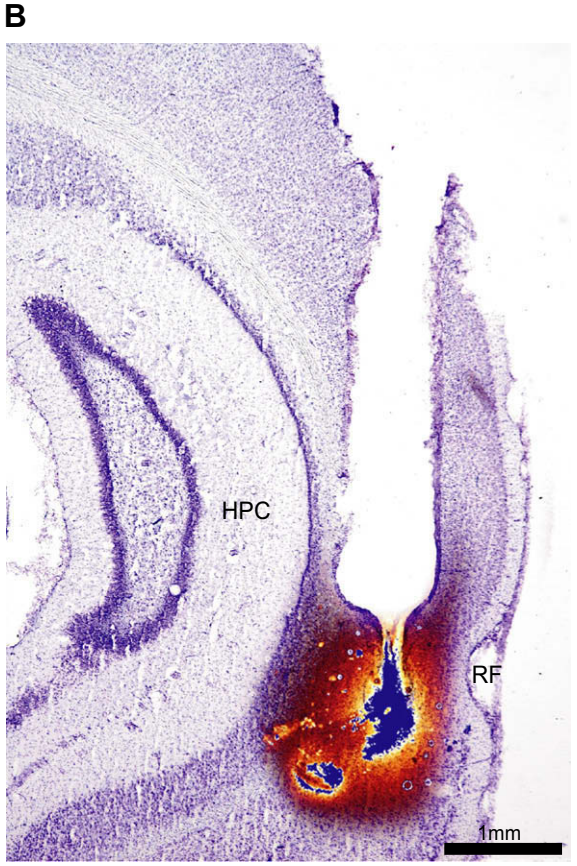


Fig. 2. (A) Schematic representation of the infusion cannula tip placements for all rats from Experiments 1 and 2 ($n = 30$). Cannulas were consistently located between 5.80 and 6.30 mm posterior to bregma. Some cannula tips overlap in the figure. (B) Representative section from the brain of a rat infused with 1 μl of fluorophore-conjugated muscimol and perfused 15 min later showing the typical region of diffusion within PRh. The fluorescent signal has been superimposed onto an adjacent cresyl violet-stained section from the same animal. The section corresponds to approximately 6.2 mm posterior to bregma (Paxinos & Watson, 1998). HPC, hippocampus; RF, rhinal fissure.

all infused rats, FCM was observed in an area extending from approximately 3.1 mm posterior to bregma (the rostral border of PRh) to approximately 7.64 mm posterior to bregma, and the fluorescent signal appeared much stronger closer to the origin of the infusion. As Fig. 2B indicates, there was no sign of significant diffusion of FCM into area TE. Although some minor spread into subjacent entorhinal cortex was observed with the larger volume infusions, this was not a consistent bilateral finding, and the highest magnitude signal was clearly centered in PRh in all brains examined. Indeed, PRh was the only area consistently and bilaterally labeled throughout the diffusion area in all brains analyzed. There are also some signs of minor mechanical damage at the site of infusion in Fig. 2B. This is typical of the mechanical damage observed in brain slices from animals tested in the visual discrimination task. Extensive damage was not observed in the experimental animals, and the damage that was seen was limited to the area in the direct vicinity of the infusion cannula tip. Moreover, this same damage was seen in the control animals infused with saline and, therefore, cannot account for the behavioral impairments reported.

3.2. Experiment 1: transient inactivation of PRh with muscimol disrupts acquisition of a two-choice visual discrimination task

Fig. 3, which shows the percent correct for each group across five training blocks of two sessions each, illustrates that rats that received daily intra-PRh infusions of muscimol were impaired relative to saline-infused rats in acquisition of the two-choice visual discrimination task. Repeated-measures ANOVA revealed significant overall effects of drug ($F_{(1, 11)} = 11.7$, $p < 0.01$) and session ($F_{(9, 99)} = 7.28$, $p < 0.001$), but the interaction failed to reach significance ($F_{(9, 99)} = 1.79$, $p = 0.08$). Analyses of the control measures revealed no significant differences between groups in terms of the average number of trials completed ($t < 1$; means ± SEM: saline = 55.68 ± 1.62, muscimol = 52.2 ± 4.02), average response latency ($t_{(11)} = 1.93$, $p = 0.08$; saline = 2.49 ± 0.23 s, muscimol = 3.67 ± 0.58 s), or average magazine latency ($t_{(11)} = 1.47$, $p = 0.17$; saline = 0.98 ± 0.05 s, muscimol = 1.17 ± 0.12 s) over the 10 training sessions.

3.3. Experiment 2: transient blockade of muscarinic or NMDA receptors in PRh disrupts two-choice visual discrimination acquisition

Rats that received daily intra-PRh infusions of either scopolamine or AP5 were impaired relative to saline-infused rats in acquisition of

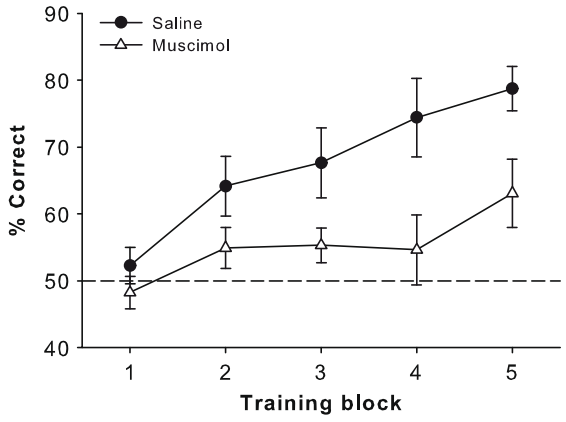


Fig. 3. Acquisition of the two-choice visual discrimination task in Experiment 1. Rats were trained on the task for 10 sessions with daily pre-session infusions of saline or muscimol into PRh. Data are presented in five two-session training blocks. The group mean (±SEM) is given for each training block.

the two-choice visual discrimination task (Fig. 4). Repeated-measures ANOVA revealed significant overall effects of drug ($F_{(2, 14)} = 5.18$, $p < 0.05$) and session ($F_{(9, 126)} = 13.54$, $p < 0.001$), but the interaction was not significant ($F_{(18, 126)} = 1.13$, $p = 0.33$). Newman–Keuls post hoc analysis revealed that both the scopolamine- and AP5-infused groups were significantly impaired compared to the saline group (both $p < 0.05$). Analyses of the control measures revealed no significant differences between groups in terms of the average number of trials completed ($F_{(2, 14)} = 1.85$, $p = 0.19$; means ± SEM: saline = 46.32 ± 6.5, AP5 = 52.67 ± 2.95, scopolamine = 54.82 ± 1.75), average response latency ($F_{(2, 14)} = 3.28$, $p = 0.07$; saline = 3.92 ± 0.79 s, AP5 = 3.68 ± 1.37 s, scopolamine = 3.77 ± 0.46 s), or average magazine latency ($F < 1$; saline = 1.44 ± 0.29 s, AP5 = 1.07 ± 0.04 s, scopolamine = 1.37 ± 0.06 s) over the 10 training sessions.

4. Discussion

The present results indicate involvement of PRh in the acquisition of a two-choice visual discrimination task. Rats given daily pre-session saline infusions required 10 sessions to achieve approximately 80% correct performance as a group in this task, whereas rats given daily pre-session muscimol achieved scores of only approximately 60% correct after the same number of sessions. The acquisition impairment caused by daily intra-PRh infusions of muscimol shows that PRh neuronal activity is necessary for normal two-choice visual discrimination learning. Moreover, daily pre-session intra-PRh infusions of scopolamine or AP5 disrupted task acquisition, indicating roles for both muscarinic cholinergic and NMDA receptors within PRh. It is likely that these effects are due to receptor blockade confined to PRh, as our previous studies have shown that radiolabeled lidocaine infused into PRh using similar methods resulted in spread of infusate which did not extend beyond the boundaries of PRh (Winters & Bussey, 2005c). The analysis of fluorescent muscimol spread in the current study is consistent with these findings; there was no indication that drug administration by the parameters used in the current study was associated with any significant infusate spread into areas adjacent to PRh, such as the hippocampus, entorhinal cortex, or area TE. The present results are consistent with similar findings from studies using one-trial object recognition memory tasks (Barker, Warburton, et al., 2006; Tang, Mishkin, & Aigner, 1997; Warburton et al., 2003; Winters & Bussey, 2005a, 2005b; Winters et al., 2006) and strongly suggest that the cognitive functions of PRh extend beyond object recognition memory to contribute to visual discrimination learning, at least when complex visual stimulus processing is required. These findings imply a generality of PRh involvement in different types of learning tasks. The two-choice visual discrimination assessed in the present study is acquired gradually, with animals eventually reaching a learning plateau, whereas the SOR task assesses the ability of rats to recognize an object after only a single viewing. The now well-established role of PRh in complex visual stimulus processing may help to explain its involvement in both types of learning and memory tasks.

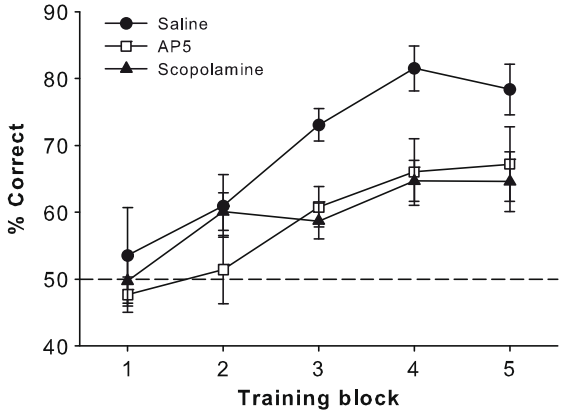


Fig. 4. Acquisition of the two-choice visual discrimination task in Experiment 2. Rats were trained on the task for 10 sessions with daily pre-session infusions of saline, AP5, or scopolamine into PRh. Data are presented in five two-session training blocks. The group mean (±SEM) is given for each training block.

Previous studies, primarily in brain-damaged monkeys, have implicated PRh in the acquisition and performance of visual discrimination tasks (Baxter & Murray, 2001; Buckley & Gaffan, 1997; Bussey et al., 2002, 2003; Eacott et al., 2001). A consistent finding is that this involvement is related to the role of PRh in complex perceptual processes related to object identification. For example, Bussey et al. (2003) showed that monkeys with PRh lesions were impaired at acquiring two-choice visual discriminations only when the visual stimuli were 'morphed' together sufficiently to yield a relatively high degree of perceptual difficulty caused by overlapping features. The easier and more quickly learned ('unmorphed') version of the task was not affected by PRh lesions, strongly suggesting that the role of PRh in this task is related to perceptual function. Thus, the present findings are consistent with previous results indicating a role for PRh in two-choice visual discrimination acquisition and performance. Furthermore, our findings extend these past results by introducing a potentially highly valuable rodent version of the touchscreen-based visual discrimination task and showing that similar learning deficits are seen in rats with even subtler brain manipulations.

The involvement of PRh in the visual discrimination task used in our study may be, at least partly, due to its perceptual functions. The visual stimuli used in the present study, although not 'morphed' together, were photographic images, the complexity of which may have necessitated the use of configural representations stored in PRh (Bussey & Saksida, 2007; Murray & Bussey, 1999; Murray et al., 2007). In the monkey study (Bussey et al., 2003), morphing may have been required to produce sufficient perceptual difficulty to tax the monkeys' superior visual system, whereas the complexity of the images used in the present study might have placed sufficient demands on rats without morphing. Past findings support the view that PRh is anatomically well-placed to process certain types of information about the identity of objects and complex visual stimuli (Bartko, Winters, Cowell, Saksida, & Bussey, 2007a; Buckley, Booth, Rolls, & Gaffan, 2001; Buckley & Gaffan, 1998; Bussey et al., 2002, 2003; Lee et al., 2005; Lee, Scahill, & Graham, 2007; Norman & Eacott, 2004). Thus, PRh may be important for the specific version of two-choice visual discrimination used in the present study because of the stimulus materials used. This proposal is consistent with recent suggestions that a hard distinction between memory and perceptual "systems" may not be the most constructive way of thinking about the functional organization of the cortex (Bussey & Saksida, 2007; Gaffan, 2001, 2002; Murray et al., 2007). Rather, there is now abundant evidence that certain cortical regions are essential for both memory and perception and may be "specialized" only in the sense that they are anatomically well-adapted to processing certain kinds of information (Bussey & Saksida, 2007; Gaffan, 2002). PRh seems to be specialized to process information about objects and other complex stimuli, and this characteristic might explain its involvement in visual discrimination tasks like the one used in the present study (see also, Graham et al., 2006).

The touchscreen-based rat visual discrimination task used in the present study has several potential advantages over monkey models, including the ability to run high throughput experiments more practically. Furthermore, highly specific manipulations can be performed more readily in rats to study the neural bases of vi-

sual discrimination learning. For example, the present results extend previous findings by demonstrating that two-choice visual discrimination learning is mediated by muscarinic cholinergic and NMDA receptors in PRh. The same neurotransmitter receptors are known to be involved in PRh-mediated object recognition memory. This generality supports the view that PRh is involved in these tasks because of a basic role in visual stimulus representation that is useful for a range of cognitive tasks. Daily pre-training intra-PRh infusions of the muscarinic cholinergic receptor antagonist scopolamine significantly disrupted task acquisition. Recent research has implicated PRh muscarinic receptors in the acquisition of object information in the SOR task (Winters, Bartko, Saksida, & Bussey, 2007; Winters et al., 2006), as well as PRh long-term depression, which might underlie object recognition memory in PRh (Warburton et al., 2003). We have previously suggested, consistent with others (Everitt & Robbins, 1997; Hasselmo & McGaughy, 2004; Sarter & Bruno, 1997), that acetylcholine in PRh might enhance cortical information processing to facilitate the acquisition of new information in the object recognition task (Winters et al., 2006, 2007). A similar process could mediate PRh involvement in the two-choice visual discrimination task. Indeed, the finding that daily pre-training intra-PRh infusions of AP5 disrupted two-choice visual discrimination acquisition supports the proposal of an associative role for PRh in this task and is consistent with suggestions that the same 'Hebbian' learning mechanism might mediate both the perceptual and mnemonic functions of PRh (Cowell, Bussey, & Saksida, 2006). Previous studies have implicated PRh NMDA receptors in the acquisition of object information required for object recognition memory (Barker, Warburton, et al., 2006; Winters & Bussey, 2005a), and the present findings suggest that NMDA receptors in PRh play a similar role in visual discrimination learning. Although these results suggest strong commonalities between PRh involvement in object recognition memory and visual discrimination learning, further research will be required to determine whether muscarinic and NMDA receptors contribute to these two types of learning through the same mechanisms and to assess the possibility of further mechanistic differences between PRh contributions to these two tasks.

The effects reported in the present study were limited to choice accuracy, with no significant effects on response latencies or latencies to collect reward. However, as noted by an anonymous reviewer, there were non-significant trends in both experiments for drug effects on the average response latency measure. It is possible that larger group sizes may have revealed significant effects on response latencies in both experiments. However, it is highly unlikely that even significant drug effects on response latencies in the directions of the trends seen in the two experiments would easily explain the significant effects on choice accuracy, because the drug-induced accuracy deficits were associated across experiments with both increased (Experiment 1 – muscimol) and decreased (Experiment 2 – scopolamine/AP5) response latencies. Thus there was no relationship between trends in response latencies and effects on choice accuracy.

It also seems unlikely that the acquisition deficits in the present study can be fully accounted for by drug-induced disruption of attention. Although one recent study has implicated PRh in attentional orienting to a conditioned stimulus (a light) (Bucci & Burwell, 2004), the fact that response and magazine latencies were not significantly altered by any of the drug treatments, showing that manipulation of PRh does not affect the animals' ability to detect and respond to stimuli, suggests that attention was not significantly affected. Indeed, as mentioned above, the trends in response latencies were in opposite directions, although the impairments in learning were the same. Moreover, intra-PRh scopolamine and AP5 administration significantly disrupted choice accuracy despite slightly decreasing response latencies, a result suggestive of, if anything, enhanced attentional orienting towards the behaviorally relevant stimuli. Finally, it is difficult to see how an impairment in conditioned orienting can account for the many reported effects of PRh manipulations (Barker, Warburton, et al., 2006; Bartko et al., 2007a; Bartko, Winters, Cowell, Saksida, & Bussey, 2007b; Buckley et al., 2001; Buckley & Gaffan, 1997, 1998; Bussey et al., 2002, 2003; Meunier et al., 1993; Saksida, Bussey, Buckmaster, & Murray, 2007; Warburton et al., 2005, 2003; Winters & Bussey, 2005a, 2005c; Winters et al., 2004), effects which can all be accommodated by the representational view advocated here.

There is some reason to suggest that PRh may only be required for early stages of acquisition in the two-choice visual discrimination tested here. In fact, there is much evidence for the temporal reorganization of the neural substrates of certain types of learning and memory tasks (Bontempi, Laurent-Demir, Destrade, & Jaffard, 1999; Frankland & Bontempi, 2005; Frankland, Bontempi, Talton, Kaczmarek, & Silva, 2004), and many tasks may be soluble by multiple strategies, which likely depend on different neural substrates (Chang & Gold, 2003; Packard & McGaugh, 1996; Poldrack et al., 2001). Thus, for example, a spatial discrimination task may rely on hippocampus-dependent place learning strategies in early stages of acquisition, but later stages may be mediated by striatum-dependent habit-like response strategies (Packard & McGaugh, 1996). In both of the current experiments, drug-treated rats may have been showing signs of learning towards the end of the testing period. In all three cases, however, at the end of testing the groups receiving PRh infusions had not acquired the task beyond chance-level performance. Thus, although we cannot conclude that these animals would never have acquired the task, we can conclude that all three manipulations significantly impaired some aspect of visual discrimination learning.

To conclude, the present findings indicate an important role for PRh, as well as muscarinic and NMDA receptors within PRh, in two-choice visual discrimination learning. These results complement recent reports of similar PRh and receptor involvement in object recognition memory. It is notable that the same anatomical substrate and neurotransmitter receptors should underlie performance in these two rather different learning and memory tasks. These findings together are suggestive of a mechanistic generality in the PRh-mediated processing of information for object recognition memory and visual discrimination learning. The common involvement of PRh muscarinic and NMDA receptors in both types of tasks may be related to the role of PRh in visual stimulus processing and representation.

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