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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,