Neurobiology of Learning and Memory 90 (2008) 616–623

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Intra-amygdala anxiogenic drug infusion prior to retrieval biases rats towards the use of habit memory

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ARTICLE INFO

Article history:
Received 14 May 2008
Revised 24 June 2008
Accepted 25 June 2008
Available online 2 September 2008

Keywords:
Amygdala
Emotion
Anxiety
Hippocampus
Striatum
Memory
RS 79948-197-197
Basal ganglia
Place learning
Response learning
Post traumatic stress disorder
Addiction

ABSTRACT

In a dual-solution plus-maze task that can be acquired using either hippocampus-dependent "cognitive/place" learning or dorsal striatal-dependent "habit/response" learning, *pre-acquisition* peripheral or intra-basolateral amygdala (BLA) injections of anxiogenic drugs result in the predominant use of response learning. The present experiments examined the effect of anxiogenic drug treatment on the relative use of multiple memory systems when administered prior to memory *retrieval*. Adult male Long-Evans rats were trained for two days (6 trials/day, 30 s ITI) in a dual-solution plus-maze task to swim from the same start point (south) to an escape platform that was located in a consistent goal arm (west). On day three, prior to a memory retrieval probe trial from a novel start point (north), rats received a peripheral (0.03, 0.1 or 0.3 mg/kg), or intra-BLA (0.1 μg/0.5 μl) injection of the anxiogenic α_2-adrenoreceptor antagonist RS 79948-197, or saline. Relative to saline controls, rats receiving either peripheral or intra-BLA infusions of RS 79948-197 predominantly displayed response learning on the probe trial. In an additional experiment peripheral (0.1 mg/kg) or intra-BLA (0.1 μg) drug injections administered prior to both acquisition *and* retrieval also resulted in the predominant use of response learning. The findings indicate that (1) similar to acquisition, peripheral injection of an anxiogenic drug prior to memory retrieval biases rats towards the use of habit/response memory, (2) intra-BLA infusions of an anxiogenic drug is *sufficient* to produce this modulatory effect of emotional state on memory retrieval, and (3) state-dependency does not appear to play a role in the effects of anxiogenic drug treatment on multiple memory system use. The findings may have implications for understanding the interaction between brain function, emotion, and the relative use of multiple memory systems in human psychopathology.

1. Introduction

Converging evidence from studies employing brain lesions (e.g., Packard, Hirsh, & White, 1989; Packard & McGaugh, 1992; Packard & McGaugh, 1996; Kesner, Bolland, & Dakis, 1993) and post-training intracerebral drug administration in rats (e.g., Packard, 1999; Packard & Teather, 1997; Packard & White, 1991) support the hypothesis that the hippocampus and dorsal striatum selectively mediate cognitive (Tolman, 1932) and stimulus-response habit (Hull, 1943) learning, respectively. A third brain structure, the basolateral amygdala (BLA), has been implicated in stimulus-affect learning (for review see White & McDonald, 2002), and can modulate both hippocampus-dependent and dorsal striatal-dependent memory processes (Packard, Cahill, & McGaugh, 1994; Packard & Teather, 1998). Numerous studies have also implicated the BLA in the effects of emotional arousal on memory (for review see McGaugh, 2004). Therefore, within the context of multiple memory systems theory we previously (Packard & Wingard, 2004) investigated the relationship between BLA function, emotional state, and the *relative* use of cognitive and habit memory in a dual-solution plus-maze task. In this task, rats are trained in a water plus-maze to swim from the same start arm (south) to an escape platform located in a consistent goal arm (west). Rats can acquire this task using hippocampus-dependent "cognitive/place" learning (i.e., approach the goal arm based on knowledge of the spatial location of the platform), or dorsal striatal-dependent "habit/response" learning (i.e., turn left at the choice point and approach the platform; Chang & Gold, 2003; Packard, 1999; Packard & McGaugh, 1996; Schroeder, Wingard, & Packard, 2002). In our previous study we observed that *pre-acquisition* peripheral or intra-BLA injections of anxiogenic drugs result in the predominant use of response learning (Packard & Wingard, 2004). These findings suggest that an anxiogenic emotional state induced prior to training favors the relative use of habit memory. Identification of the neural mechanisms by which emotional arousal can produce a bias towards the use of habit memory is important in view of evidence that anxiety and/or stress can be contributing factors to relapse into previously acquired habitual and maladaptive behaviors in human psychopathology (e.g.,

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doi:10.1016/j.nlm.2008.06.012

Herman & Polivy, 1975; Weiss et al., 2001). Therefore, the primary goal of the present experiments was to extend our previous findings by examining the relative use of multiple memory systems when an anxiogenic drug is administered prior to memory *retrieval*. Accordingly, in experiment one rats trained in a dual-solution water plus-maze task were injected either peripherally or directly into the BLA with the anxiogenic α_2-adrenoreceptor antagonist RS 79948-197 or vehicle prior to memory retrieval.

A secondary goal of the present experiments was to examine whether drug-induced state dependency may play in the role in the effects of anxiogenic drug treatment on the relative use of multiple memory systems. According to the state dependency hypothesis, information is best retrieved if the organism is in the same physiological state during initial task acquisition and memory retrieval (Overton, 1964). In the context of the dual-solution plus-maze task, the state dependency hypothesis raises the possibility that in our previous study (Packard & Wingard, 2004), rats receiving injections of an anxiogenic drug prior to acquisition predominantly displayed response learning on the subsequent drug-free memory retrieval probe trial because they were not in the same physiological state at these two behavioral time-points. In order to examine this hypothesis, we conducted an additional experiment in which rats received peripheral or intra-BLA injections of RS 79948-197 prior to both acquisition *and* memory retrieval. If the predominant use of response learning produced by RS 79948-197 is due to state-dependency, then drug injections at both time-points should "restore" behavior to the same pattern as that observed in control rats. In contrast, if the effects of RS 79948-197 are not due to state-dependency, then injections at both time-points should continue to result in the predominant use of response learning.

2. Materials and methods

2.1. Subjects

Subjects were 138 male Long-Evans rats (weighing 275–350 g). They were individually housed in a climate-controlled vivarium with ad libitum access to food and water. The animals were on a 12:12-h light: dark cycle (lights on at 8 a.m.). All experiments were conducted during the light phase of the cycle.

2.2. Apparatus

Animals were trained in a black circular water maze (1.83 m diameter, 0.58 m in height; 25 degrees Celsius water-temperature) into which a clear Plexiglas plus-maze (43 cm height, arm-width of 25 cm, and arm-length of 60 cm) was inserted (Packard & Wingard, 2004). The maze was filled to a water-level of 20 cm. Extra-maze visual cues of various geometric shapes were placed on the room walls. None of the extra-cues were placed in a spatially congruent/proximal position with the ends of the plus-maze arms. During training, an invisible clear Plexiglas escape platform ($11 \times 14 \times 19$ cm) was consistently located at the end of one arm of the maze (west), 1cm below water level. The arm opposite the start-arm was blocked off by an additional piece of Plexiglas, such that the rats were trained with the maze in a "T" configuration.

2.3. Surgery and histology

Rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg) and implanted with bilateral guide cannula in the basolateral amygdala using standard stereotaxic techniques. Guide cannulae (23 gauge, 15 mm length) were anchored to the skull with the jeweler's screws and dental acrylic. Stereotaxic coordinates for the basolateral amygdala were anterior-posterior (AP) = −2.2 mm from bregma, medial-lateral (ML) = +4.7 mm, and dorsal-ventral (DV) = −7.00 mm. These coordinates were chosen based on our previous research (Packard & Teather, 1998; Packard & Wingard, 2004). Animals were given 7–10 days of post-operative recovery prior to behavioral testing.

Following behavioral testing, rats were deeply anesthetized with a 1 ml-injection of sodium pentobarbital (60 mg/kg) and perfused with 0.9% saline, followed by 10% formal-saline solution. The brains were removed and stored in 10% formal-saline solution before slicing with a cryostat. Brains were sectioned at 20 lm and every second slice was collected and stained with cresyl violet. Slides were examined for cannula placements and infusion needle tip location using the atlas of Paxinos and Watson (1997). Rats with inaccurate cannula placements ($n = 7$) were excluded from the statistical analysis. As illustrated in Fig. 1, the injection needle tips were located in the basolateral amygdala ranging from −1.80 mm to −2.80 mm AP from bregma.

It should be noted that although the tip of the injection needles were located in the BLA, the possibility of the injection spreading to other amygdala nuclei (e.g., central nucleus) cannot be completely ruled out. However, pharmacological and electrical evidence indicates that the memory modulatory functions of the amygdala involve the BLA and not the central nucleus (e.g., Akirav & Richter-Levin, 2002; Roozendaal & McGaugh, 1996; Roozendaal & McGaugh, 1997). In addition, the dual-solution plus maze task used in the present study engages hippocampus-dependent and dorsal striatal-dependent learning (e.g., Packard & McGaugh, 1996), and the BLA projects directly to both of these structures

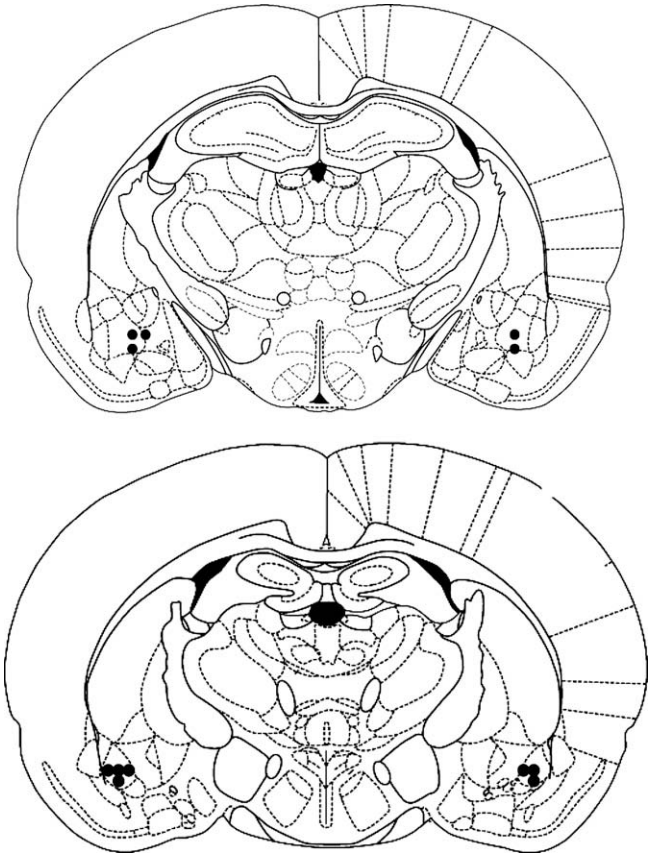

Fig. 1. Location of bilateral injection needle tips in the basolateral amygdala (shown with overlap). Infusions needles were located in the basolateral nucleus ranging from −1.80 to −2.80 mm AP from bregma. Adapted from the rat brain atlas of Paxinos and Watson, Figure 31 (1997).