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# Exposure to predator odor influences the relative use of multiple memory systems: Role of basolateral amygdala

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ABSTRACT

In a dual-solution plus-maze task in which both hippocampus-dependent place learning and dorsolateral striatal-dependent response learning provide an adequate solution, the relative use of multiple memory systems can be influenced by emotional state. Specifically, pre-training peripheral or intra-basolateral (BLA) administration of anxiogenic drugs result in the predominant use of response learning. The present experiments were designed to extend these findings by examining whether exposure to a putatively ethologically valid stressor would also produce a predominant use of response learning. In experiment 1, adult male Long-Evans rats were exposed to either a predator odor (trimethylthiazoline [TMT], a component of fox feces) or distilled water prior to training in a dual-solution water plus maze task. On a probe trial 24 h following task acquisition, rats previously exposed to TMT predominantly displayed response learning relative to control animals. In experiment 2, rats trained on a single-solution plus maze task that required the use of response learning displayed enhanced acquisition following pre-training TMT exposure. In experiment 3, rats exposed to TMT or distilled water were trained in the dual-solution task and received post-training intra-BLA injections of the sodium channel blocker bupivacaine (1.0% solution, 0.5 μl) or saline. Relative to control animals, rats exposed to TMT predominantly displayed response learning on the probe trial, and this effect was blocked by neural inactivation of the BLA. The findings indicate that (1) the use of dorsal striatal-dependent habit memory produced by emotional arousal generalizes from anxiogenic drug administration to a putatively ecologically valid stressor (i.e. predator odor), and (2) the BLA mediates the modulatory effect of exposure to predator odor on the relative use of multiple memory systems.



## 1. Introduction

Several neurobehavioral studies employing the use of reversible and irreversible lesion techniques in animals have demonstrated double dissociations between the roles of the hippocampal system and the dorsal striatum in "cognitive" and stimulus–response "habit" learning and memory, respectively (for reviews see Packard & Knowlton, 2002; White & McDonald, 2002). For example, in a dual-solution plus-maze task that can be acquired using either place or response learning, neural inactivation of the hippocampus impairs place learning, whereas inactivation of the dorsolateral striatum impairs response learning (Packard & McGaugh, 1996; for review see Packard, 2009a).

In view of evidence that multiple memory systems can be activated in parallel, recent studies have focused on potential factors that may influence the *relative* use of these memory systems in a given learning situation. One factor that been examined in this context is the memory modulatory effect of emotional arousal, specifically in the form of stress and/or anxiety. For example, in a dual-solution plus-maze task, peripheral pre-training administration of anxiogenic drugs (i.e. noradrenergic alpha-2 receptor antagonists yohimbine or RS 79948-197) can bias rats towards the use of dorsolateral striatal-dependent response learning (Packard & Wingard, 2004). Consistent with evidence that the basolateral amygdala (BLA) exerts a memory modulatory influence that is linked to emotional arousal (for review see McGaugh, 2004), intra-BLA injections of an anxiogenic dose of RS-79948-197 also produces a predominant use of response learning (Packard & Wingard, 2004; Wingard & Packard, 2008).

Whereas previous research investigated the effects of emotional arousal on the relative use of multiple memory systems by employing pharmacological manipulations (i.e. anxiogenic drug treatment), the present experiments were designed to extend these findings by employing exposure to predator odor, a putatively ethologically valid stressor. It is well established that exposure to predator odor induces stress and anxiety in rodents (Griffith, 1919, 1920). Rats that have been exposed to cat fur/odor exhibit a range of fear/anxiety-like avoidance behaviors such as

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freezing, hiding, and decreased stimulus contact (Blanchard, Blanchard, Wiess, & Meyers, 1990; Dielenberg & McGregor, 2001). In addition, exposure to 2,5-dihydro-2,4,5-trimethylthiazoline (TMT), a sulfur-containing compound that is specific to red fox feces, also induces fear/anxiety in laboratory rats (Burwash, Tobin, Woolhouse, & Sullivan, 1998; Morrow, Elsworth, & Roth, 2002; Vernet-Maury, 1980; Vernet-Maury, Polak, & Demael, 1984; for review see Fendt, Endres, Lowry, Apfelbach, & McGregor, 2005). Several studies have examined the effect of exposure to TMT on performance of various learning and memory tasks (for review see Takahashi, Nakashima, Hong, & Watanabe, 2005) and TMT has been used extensively as an unconditioned stimulus (US) in fear conditioning tasks (for review see Takahashi, Chan, & Pilar, 2008).

In experiment 1 we examined the effect of pre-training exposure to TMT on the relative use of "place" and "response" learning in a dual-solution water plus-maze. In this task rats are trained to swim from the same start arm to a hidden escape platform that is always located in the same goal arm. This dual-solution task can be acquired by learning to make the same body turn response at the choice point (i.e. response learning), or alternatively by learning the spatial location of the escape platform (i.e. place learning). The relative use of these strategies can be assessed after acquisition on a probe trial in which rats are started from the opposite start arm. On the probe trial, rats that swim to the same spatial location in which the platform was located during training are designated "place" learners, whereas rats that make the body turn response reinforced during training are designated "response" learners". Response learning in the plus-maze is dorsolateral striatal-dependent, whereas place learning is hippocampus-dependent (Packard, 1999; Packard & McGaugh, 1996; for review see Packard, 2009b).

In experiment 2 we investigated the effect of pre-training TMT exposure on acquisition of a dorsolateral striatal-dependent single-solution plus-maze task that *required* the use of response learning. In this task, rats are started from varying start arms (North, South) and are trained to always make the same body turn response (e.g. turn left) at the maze choice point. Finally, in experiment 3 we examined the effect of post-training neural inactivation of the BLA on the ability of TMT exposure to influence the relative use of place and response learning.

## 2. General methods

### 2.1. Subjects

Subjects ($n = 98$) were experimentally naïve adult male Charles River Long-Evans rats (weighing 300–400 g). Animals were housed individually in a climate-controlled vivarium with *ad libitum* access to food and water. Experiments were conducted during the light phase cycle of a 12:12 h light–dark cycle (lights on at 7 a.m.).

### 2.2. Apparatus

The water plus-maze apparatus used was identical to that used in our previous studies (e.g. Leong, Goodman, & Packard, 2012; Packard & Gabriele, 2009). A clear Plexiglas plus-maze (43.2 cm in height, 26 cm in arm-width, and 59.1 cm in length) was inserted into a black circular water maze (1.73 m in diameter, 45 cm in height). The maze was filled with water (25 °C) to a depth of approximately 21 cm. A submerged Plexiglas escape platform ($14 \times 14 \times 20$ cm) was located at the end of a maze arm that varied depending on the specific training protocol. The PVC holding containers used for either predator odor or distilled water exposure ($45 \times 30 \times 25$ cm) was placed underneath a ventilation hood.

### 2.3. Odorant exposure and drugs injections

The method of exposure to the predator odor 2,3,5-trimethyl-3-thiazoline (TMT) was similar to previous studies (Endres & Fendt, 2008; Fendt, Endres, & Apfelbach, 2003). Consistent with several previous studies examining the behavioral effects of TMT exposure (e.g. Galliot, Levaillant, Beard, Millot, & Pourie, 2010; Hacquemand, Jacquot, & Brand, 2012; Morrow, Roth, & Elsworth, 2000) distilled water was used for the control group. TMT (5 μl) or distilled water (5 μl) was deposited onto circular filter paper (4.7 cm diameter) and placed on the wall of the holding container 10 cm from the bottom. Rats were placed into the appropriate TMT or control (distilled water) container for 5 min immediately prior to training.

In experiment 3, bilateral intra-BLA infusions (0.5 μl/side) of bupivicaine (1% solution, Abbott Laboratories) or saline were administered via a microsyringe pump with an electronic timer (Sage Instruments) through 10 μl Hamilton syringes connected to an polyethylene tubing (PE 10) and injection needle (16 mm length, 30 gauge). Bupivicaine acts as a sodium channel blocker, hence providing temporary inactivation of the region via the blockade of action potential conductance. Infusions were administered over a period of 52 s. Following this period, injection needles were left in the guide cannula for an additional 60 s to allow for diffusion.

### 2.4. Surgery

Prior to surgery rats were anesthetized with vapor isoflurane and then implanted with bilateral guide cannulae using standard stereotaxic procedure. The guide cannulae (23 gauge, 15 mm in length) were inserted into the brain overlying the BLA and held in place using jeweler's screws and dental acrylic. The stereotaxic coordinates for the BLA guide cannulae placements were AP = −2.2 mm, ML = ±4.7 mm, DV = −7.0 mm. These coordinates are the same as our previous studies that have examined the role of intra-BLA drug infusions on plus-maze behavior (e.g. Packard & Gabriele, 2009; Packard & Wingard, 2004; Wingard & Packard, 2008). Following surgery animals were given 8 days of post-operative recovery prior to beginning behavioral training.

### 2.5. Histology

Following the completion of behavioral procedures rats were sacrificed with a 1 ml injection of pentobarbital sodium and phenytoin sodium (Euthasol Euthanasia Solution, Virbac Corporation, Texas). Rats were then perfused with physiological saline followed by 10% formaldehyde-saline solution. Brains were removed and sectioned at 20 μm through the cannula tract region using a cryostat, and were subsequently mounted on slides and stained with cresyl violet. The location of the injection needle tips were confirmed using a standard rat brain atlas (Paxinos & Watson, 1997), and were located in the basolateral amygdala ranging from −1.80 to −3.14 mm from bregma (Fig. 1). Five animals were removed from the data analyses due to cannula placements that were located outside of the BLA.

It should be noted that although the injection needle tips were located in the BLA, the possibility of drug spread into other amygdala nuclei (e.g. central nucleus) cannot be completely ruled out. Nonetheless, converging evidence suggests that the memory modulatory effects of emotional arousal are mediated by the BLA. Thus, lesions of the BLA, but not the central nucleus block amygdala influences on hippocampus-dependent memory (Roozendaal & McGaugh, 1996). In addition, post-training drug administration modulates memory when infused into the BLA, but not the central nucleus (Roozendaal & McGaugh, 1997). Finally, the influence of the amygdala on hippocampal long-term potentiation is mediated