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Enhancement of striatum-dependent memory by conditioned fear is mediated by beta-adrenergic receptors in the basolateral amygdala

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ABSTRACT

Emotional arousal can have a profound impact on various learning and memory processes. For example, unconditioned emotional stimuli (e.g., predator odor or anxiogenic drugs) enhance dorsolateral striatum (DLS)-dependent habit memory. These effects critically depend on a modulatory role of the basolateral complex of the amygdala (BLA). Recent work indicates that, like unconditioned emotional stimuli, exposure to an aversive conditioned stimulus (CS) (i.e., a tone previously paired with shock) can also enhance consolidation of DLS-dependent habit memory. The present experiments examined whether noradrenergic activity, particularly within the BLA, is required for a fear CS to enhance habit memory consolidation. First, rats underwent a fear conditioning procedure in which a tone CS was paired with an aversive unconditioned stimulus. Over the course of the next five days, rats received training in a DLS-dependent water plus-maze task, in which rats were reinforced to make a consistent body-turn response to reach a hidden escape platform. Immediately after training on days 1–3, rats received post-training systemic (Experiment 1) or intra-BLA (Experiment 2) administration of the β-adrenoreceptor antagonist, propranolol. Immediately after drug administration, half of the rats were re-exposed to the tone CS in the conditioning context (without shock). Post-training CS exposure enhanced consolidation of habit memory in vehicle-treated rats, and this effect was blocked by peripheral (Experiment 1) or intra-BLA (Experiment 2) propranolol administration. The present findings reveal that noradrenergic activity within the BLA is critical for the enhancement of DLS-dependent habit memory as a result of exposure to conditioned emotional stimuli.

1. Introduction

Mammalian memory is organized into dissociable neural systems that differ in terms of the type(s) of memory they mediate (White and McDonald, 2002; Squire, 2004; White et al., 2013). Extensive evidence indicates that among these memory systems is a stimulus-response/habit system principally dependent on the integrity of the dorsolateral striatum (DLS) (Packard et al., 1989; Packard and McGaugh, 1996; Packard and Knowlton, 2002; Yin et al., 2004; Goodman and Packard, in press). DLS-dependent memory processes have been implicated in a variety of learning and memory tasks including response learning in the plus-maze, whereby animals acquire an egocentric turning response at the maze choice-point to receive reinforcement (Packard and McGaugh, 1996; Chang and Gold, 2004; Yin and Knowlton, 2004). Memory in DLS-dependent maze tasks may be considered an exemplar of habit memory, given that the learned behavior in these tasks remains insensitive to reward devaluation (Sage and Knowlton, 2000; Lin and Liao, 2003; De Leonibus et al., 2011; Smith et al., 2012; Smith and Graybiel, 2013).

Stress influences a wide variety of learning and memory processes, and whether stress enhances or impairs memory partly depends on the type of memory being investigated (Kim and Diamond, 2002; McGaugh, 2004; Sandi and Pinelo-Nava, 2007; Packard, 2009; Roozendaal et al., 2009; Sandi, 2013; Arnsten, 2015). Converging evidence indicates that DLS-dependent habit memory in the plus-maze may be facilitated by the induction of emotional arousal through the exposure of animals to aversive unconditioned stimuli (Packard, 2009; Packard and Goodman, 2012, 2013; Sandi, 2013; Schwabe, 2013). For example, DLS-dependent habit memory may be facilitated following chronic restraint stress, tail shock,

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exposure to predator odor, or administration of anxiogenic drugs (Kim et al., 2001; Packard and Wingard, 2004; Wingard and Packard, 2008; Elliott and Packard, 2008; Schwabe et al., 2010c; Packard and Gabriele, 2009; Leong et al., 2012; Leong and Packard, 2014; Taylor et al., 2014; Goodman et al., 2015). Furthermore, some evidence suggests that, as observed with unconditioned emotional stimuli, exposure to emotionally arousing conditioned stimuli also modulates memory (Holahan and White, 2002, 2004; Hawley et al., 2013; Leong et al., 2015). In particular, recent work from our laboratory revealed that exposing rats to shock-associated stimuli (i.e., a tone and context previously paired with footshock—hereafter termed 'CS exposure') enhanced DLS-dependent habit memory and biased animals toward the use of a response learning strategy in the plus-maze (Leong et al., 2015). The neural mechanisms underlying this behavioral effect have yet to be fully characterized.

Noradrenergic activity, particularly within the basolateral complex of the amygdala (BLA), plays a critical role in regulating emotional arousal and the emotional modulation of memory (McGaugh, 2004; Roozendaal et al., 2009). Additionally, the BLA is required for the acquisition and expression of Pavlovian fear conditioning (Campeau and Davis, 1995; Maren et al., 1996; LeDoux, 2000, 2003; Maren, 2001a, 2001b). Studies have found that noradrenaline administered directly into the BLA modulates memory consolidation, whereas administration of a β-adrenoceptor antagonist blocks the emotional modulation of memory (Liang et al., 1990; Hatfield and McGaugh, 1999). In addition, the memory modulatory effects of systemically administered adrenaline are also blocked after intra-BLA administration of the β-adrenoceptor antagonist, propranolol, across a range of learning and memory tasks (Liang et al., 1986; for review, see Roozendaal et al., 2009). Evidence from our laboratory indicates that similar neural mechanisms underlie the emotional enhancement of DLS-dependent habit memory in the plus-maze. For example, administration of anxiogenic drugs directly into the BLA is sufficient to enhance DLS-dependent habit memory and the enhancement of habit memory produced by exposure to predator odor or systemic administration of anxiogenic drugs is blocked by neural inactivation of the BLA (Elliott and Packard, 2008; Wingard and Packard, 2008; Packard and Gabriele, 2009; Leong and Packard, 2014).

In view of this evidence, we hypothesized that the enhancement of DLS-dependent habit memory consolidation after exposure to an aversive CS (Leong et al., 2015) may also be dependent on noradrenergic activity, particularly within the BLA. In order to test this hypothesis, rats were first subjected to a standard fear conditioning paradigm (i.e., repeated tone-shock pairings). Rats were then trained in a response learning task in the water plus-maze that requires the use of DLS-dependent habit memory. Following training sessions, rats were given systemic (Experiment 1) or intra-BLA (Experiment 2) administration of propranolol immediately before CS exposure.

2. Materials and methods

2.1. Subjects

Subjects were experimentally naïve adult male Long Evans (Blue Spruce) rats, obtained from Harlan Laboratories (Indianapolis, IN), and weighing 275–375 g at the time of training. Subjects were individually housed in clear plastic cages with sawdust bedding in a climate-controlled vivarium. Standard rodent chow and water were accessible *ad libitum*. Experimenters handled rats for 1 min per day for five days prior to the start of behavioral training or surgeries. For Experiment 1, rats experienced a 12:12 light–dark cycle (lights on at 7:00 a.m. and off at 7:00 p.m.). Experiment 2 utilized a 14:10 light–dark schedule (lights on at 7:00 a.m. and off at 9:00 p.m.). All phases of behavioral training occurred during the light phase of the cycles. The Institutional Animal Care and Use Committee at Texas A&M University approved all experimental procedures.

2.2. Apparatus

For Experiment 1 and 2, fear conditioning occurred within 8 identical rodent conditioning chambers (MED Associates). These chambers were housed within external sound-attenuating cabinets in an isolated room. The chambers (30 cm × 24 cm × 21 cm) are comprised of aluminum (side walls) and Plexiglas (real wall, front door, and ceiling). The floor of each chamber consisted of 19 stainless steel rods (4 mm in diameter) spaced center to center at 1.5 cm apart. Footshock (2 s, 1 mA; unconditioned stimulus, US) was delivered via a shock source and solid-state grid scrambler (MED Associates). A speaker attached to each individual chamber provided the auditory conditioned stimulus (2 kHz, 20 s, 80 dB). Small fans in each cabinet provided background noise (70 dB). Cameras mounted above the Plexiglas ceiling of the chambers remotely recorded each animal's behavior. For the conditioning context, a small volume of 1.5% acetic acid odor was poured into the metal pan beneath the grid floor, the testing room lights remained on, and the cabinet doors were left open. Each chamber was cleaned with water and acetic acid before and after conditioning. The same contextual cues were used for both conditioning and CS exposure sessions. A load-cell platform beneath each chamber recorded chamber displacement (−10 V to +10 V) as a result of each animal's movement. Load-cell activity values were acquired and digitized at 5 Hz with Threshold Activity software (Med Associates). Activity values were transformed offline into absolute values ranging from 0 to 100 (with lower values indicating less displacement of the chamber); rats were scored as freezing if absolute values were $\leq$10 for 1 s or more. Freezing was analyzed as a percentage of total time across each trial as described below.

The water maze consisted of a clear Plexiglas plus-maze (43 cm in height; each arm is 27 cm wide and 60 cm in length) that was inserted in a black circular tub (180 cm in diameter; 45 cm in height; see Leong et al., 2012; Goodman and Packard, 2014; Leong and Packard, 2014; Leong et al., 2015). For Experiment 1 and 2, the maze was filled with water to a level of ~21 cm; water temperature was 25 °C (i.e., room temperature). A submerged clear plastic platform (15 cm × 14 cm × 20 cm) served as the hidden escape platform; the platform was about ~1 cm below the water level throughout maze training. A movable piece of Plexiglas (43 cm in height; 27 cm wide) blocked entry into the arm opposite to the start arm for each trial, creating a T-maze as necessary for the response learning task described below. The maze room contained multiple extra-maze cues.

2.3. Surgery

Prior to behavioral training in Experiment 2, rats were anesthetized with isoflurane and treated with atropine nitrate (0.4 mg/kg, i.p.). Each rat was secured in a stereotaxic frame (David Kopf Instruments) and a small incision was made in the tissue above the skull; bregma and lambda of the skull were leveled on an even plane. Jeweler's screws were affixed to the skull. Small holes were drilled in the skull and guide cannulae (10 mm, 26 gauge; Small Parts) were lowered to the following coordinates: −2.2 posterior to bregma; ±5.0 medial/lateral to the midline; −6.0 ventral to dura (targeting the BLA). Dental cement was used to anchor the guide cannulae to the screws in the skull. Stainless steel dummy cannulae (11 mm, 30 gauge) were inserted into the guide cannulae (extending 1 mm beyond the end of the guide cannulae into the