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Cholinergic transmission in the dorsal hippocampus modulates trace but not delay fear conditioning

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ABSTRACT

Although cholinergic mechanisms have been widely implicated in learning and memory processes, few studies have investigated the specific contribution of hippocampal cholinergic transmission during trace fear conditioning, a form of associative learning involving a temporal gap between two stimuli. Microinfusions of scopolamine, a muscarinic receptor antagonist, into the dorsal hippocampus (DH) produced dose-dependent impairment in the acquisition and expression of a conditioned response (CR) following trace fear conditioning with a tone conditioned stimulus (CS) and a footshock unconditioned stimulus (US) in rats. The same infusions, however, had no effect on delay conditioning, general activity, pain sensitivity or attentional modulation. Moreover, scopolamine infusions attenuated phosphorylation of extracellular signal-regulated kinase (ERK) in the amygdala, indicating that cholinergic signals in the DH are important for trace fear conditioning. Taken together, the current study provides evidence that cholinergic neurotransmission in the DH is essential for the cellular processing of CS–US association in the amygdala when the two stimuli are temporally disconnected.

1. Introduction

The central cholinergic system is considered an essential neurotransmission mechanism in various types of learning and memory tasks (Gold, 2003; van der Zee & Luiten, 1999). Cholinergic neurons originating from the basal forebrain send global projections to various areas of the brain, the majority of which have been implicated in mnemonic functions (Dutar, Bassant, Senut, & Lamour, 1995; Everitt & Robbins, 1997). Lesion or inactivation of the forebrain cholinergic system impair various cognitive abilities such as spatial memory (Janis, Glasier, Fulop, & Stein, 1998; von Linstow Roloff, Harbaran, Micheau, Platt, & Riedel, 2007), working memory (Dougherty, Turchin, & Walsh, 1998), and attentional processing (Sarter, Bruno, & Turchi, 1999). Furthermore, the memory debilitating Alzhiemer's disease is also associated with loss of the forebrain cholinergic system (Coyle, Price, & DeLong, 1983).

The cholinergic system modulates acquisition and retrieval in Pavlovian fear conditioning, a form of associative learning where an initially neutral conditioned stimulus (CS) comes to elicit fear conditioned responses (CRs) after being paired with an aversive unconditioned stimulus (US) (Davis, 1992; Fanselow & LeDoux, 1999). Systemic injections of scopolamine, an antagonist of muscarinic cholinergic receptors, dose-dependently attenuated freezing during the acquisition and retention phases of delay fear conditioning (Anagnostaras, Maren, Sage, Goodrich, & Fanselow, 1999; Rudy, 1996) and impaired acquisition and consolidation of contextual fear conditioning (Anagnostaras, Maren, & Fanselow, 1995). However, the effect of cholinergic blockade on fear conditioning is not uniformly disruptive when different behavioral paradigms are considered. For example, pre-training infusions of scopolamine into the hippocampus blocked the acquisition of fear CRs to a contextual but not to a tone CS (Gale, Anagnostaras, & Fanselow, 2001; Rogers & Kesner, 2004; Wallenstein & Vago, 2001). On the contrary, selective removal of basal forebrain cholinergic neurons did not attenuate conditioned fear to the context nor to the tone (Frick, Kim, & Baxter, 2004). Therefore, the degree of cholinergic modulation in aversive learning seems to be determined by multiple factors including the complexity of the CS and the specific brain circuit involved. Few studies, however, have examined the role of cholinergic neurotransmission in a more temporally demanding trace fear conditioning paradigm.

In a typical trace fear conditioning paradigm, the CS and US are separated by a stimulus-free trace interval. This temporal arrangement of the CS and US requires both the hippocampus and the amygdala (McEchron, Bouwmeester, Tseng, Weiss, & Disterhoft, 1998), as opposed to delay conditioning which involves contiguous stimuli and does not require the hippocampus for acquisition (Phillips & LeDoux, 1992; Shors, 2004). Specifically, NMDA-mediated neuronal plasticity is necessary for the association of temporally disparate stimuli in the trace paradigm (Huerta, Sun, Wilson, &

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Tonegawa, 2000; Ohno et al., 2006; Shors, 2004). In support, pre- and post- training infusions of the NMDA receptor antagonist, DL-2-amino-5-phosphonovaleric acid (APV), into the dorsal hippocampus impaired trace fear conditioning in rats (Quinn, Loya, Ma, & Fanselow, 2005; Seo, Pang, Shin, Kim, & Choi, 2008) and mice (Misane et al., 2005; Wanisch, Tang, Mederer, & Wotjak, 2005). However, whether the cholinergic neurotransmitter system in the DH is involved in trace fear memory processing has not been directly tested.

Based on the converging lines of evidence suggesting that the cholinergic system is involved in normal functioning of the hippocampus (Adams, Winterer, & Muller, 2004; Buzsaki, 2002; Hasselmo, 2006; Huerta & Lisman, 1995; Ovsepian, Anwyl, & Rowan, 2004), we hypothesized that inhibition of cholinergic transmission would disturb the formation of trace fear memory. Because the amygdala is critically involved in trace conditioning through its interaction with the hippocampus, we also analyzed the phosphorylation of extracellular signal-regulated kinase (ERK), a known marker of fear memory consolidation in the amygdala (Schafe et al., 2000).

2. Materials and methods

2.1. Subjects

Male Sprague–Dawley rats (Orient Bio Inc., Gyunggi-do, Korea) weighing 250–300 g were housed individually in clear Plexiglas cages. They were maintained on a reversed 12 h light/dark cycle (lights on 21:00–09:00) under temperature-controlled conditions. Food and water were provided ad libitum. All behavioral procedures were performed during the dark cycle.

2.2. Surgery for cannula implantation

Rats were deeply anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and mounted on a stereotaxic instrument (Kopf Instruments, Tujunga, CA, USA). Bilateral guide cannulae (26 gauge, Plastics One, Roanoke, VA, USA) were implanted into the dorsal hippocampus at coordinates 3.5 mm posterior, 2.5 mm lateral, and 3.2 mm ventral to the bregma (Paxinos & Watson, 1998). The cannulae were secured to the skull with stainless steel screws and dental cement. Dummy cannulae (33 gauge; Plastics One) were inserted 0.5 mm below the guide cannulae to prevent occlusion. The rats were allowed to recover for at least 7 days before subsequent experimental procedures were performed.

2.3. Drug infusions

For drug infusions, 33-gauge injection cannulae (Plastics One) connected to 10-μl Hamilton syringes (Hamilton, Reno, NV, USA) via polyethylene catheter tubing (PE-20 tubing; Plastics One) were inserted 0.5 mm below the tips of the guide cannulae. Drugs were delivered by the syringe, which was mounted on a motorized infusion pump (KD Scientific Inc., Holliston, MA). For habituation to the infusion procedure, which requires slight immobilization by the experimenter, rats were handled for 3 days prior to the procedure. Each rat was given bilateral infusions of either artificial cerebrospinal fluid (aCSF; pH 7.2–7.4) or scopolamine hydrobromide dissolved in aCSF (SCOP; 30 μg/μl; Sigma, S1875) at a rate of 0.25 μl/min for two minutes (0.5 μl per hemisphere). After drug infusion, the injection cannulae were left in place for an additional 2 min. Fear conditioning or testing was started 15 min after completion of the infusions.

For the dose-dependent response analysis, dose of the drug was varied, based on previous studies that examined the effect of intra-hippocampal scopolamine infusions: 10, 30, or 50 μg/μl (Calandreau et al., 2006; Gale et al., 2001).

2.4. Apparatus

Fear conditioning was conducted in four identical clear Plexiglas chambers (26 × 28 × 40 cm) encased inside a double-walled sound attenuating cubicle (58 × 58 × 68 cm). Illumination was provided by four red miniature bulbs attached to the upper corners of the cubicle. The grid floor of each chamber consisted of 16 stainless steel rods (5 mm in diameter, 15 mm apart) connected to a scrambled shock generator (Coulbourn Instruments, Allentown, PA) that was used to deliver the foot-shock US. A speaker (7 × 5 cm, 8 Ω) on the middle sidewall of each chamber was connected to a tone generator (Coulbourn Instruments, Allentown, PA) for delivery of the CS. An overhead video camera was mounted on the ceiling to record the behavior of the animals. All experimental procedures were controlled by LabView software and a DAQ board (NI PCI-6251, National Instruments Co., Austin, TX).

The retrieval test was conducted in a distinctively different context. The grid floor was replaced with a flat plastic floor covered with wooden shavings, and a black Plexiglas triangular insert with 60-degree angles was placed 15 cm above the floor. Illumination was provided with four blue bulbs.

The general locomotor activity of the rats in the open field test was measured by an automatic video tracking system (SmarTrack®, Smartech, Madison, WI). A square arena (77 × 77 cm) with walls (25 cm high) was placed in the center of a well-lit room and surrounded by black curtains. Background noise (60 dB) was provided by a white noise generator.

The acoustic pre-pulse inhibition (PPI) test was conducted in a sound attenuating cubicle (50 × 50 × 50 cm). The startle stimulus was delivered by two loudspeakers (11 cm in diameter, 30 W, 8 Ω) mounted above the platform. The pre-pulse tone was delivered by a small speaker (9 cm in diameter, 60 W, 8 Ω) mounted in the front. The platform was attached to a load-cell device (DACELL Co., Ltd., Chung-buk, Korea), which was connected to an amplifier and a computer with a D/A board (NI PCI-MIO-16E-4, National Instruments Co.). Rats were loosely restrained in a clear Plexiglas tube (8 × 20 cm) and placed on the platform for measurement of the acoustic startle response.

3. Behavioral procedures

Rats were handled gently and habituated to dummy cannula removal for 2 days prior to the onset of conditioning. On the conditioning day, rats were divided into two drug groups and given intra-hippocampal infusions of aCSF or scopolamine 15 min before conditioning. Following the drug infusion, the rats were placed into the conditioning chamber. After 3 min of habituation, seven CS-US pairings were presented according to the trace or delay paradigm. Specifically, the trace procedure consisted of a 15-s tone (80 dB, 4 kHz), and a 30-s trace interval followed by 1-s footshock (0.5 mA). Delay conditioning was performed in a similar manner, except that the 30-s trace interval was absent, reducing the interstimulus interval (ISI) to 15 s. The inter-trial intervals (ITI) varied randomly between 120 and 180 s (150 s on average) for both groups. Twenty-four hours after the conditioning, half of the rats from each drug group were given infusions of aCSF before the retention test, whereas the other half were given infusions of scopolamine. Therefore, rats were assigned to four groups (aCSF–aCSF, aCSF–SCOP, SCOP–aCSF and SCOP–SCOP) based on the drug types injected before conditioning and the retention test. Fifteen minutes after drug injection, rats were placed in a distinctive context and received three tone-only trials after the 3-min habituation period.