Neurobiology of Learning and Memory 91 (2009) 93–97

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

Brief Report

Involvement of the basolateral amygdala in muscarinic cholinergic modulation of extinction memory consolidation ☆

Mariano M. Boccia [b], Mariano G. Blake [b], Carlos M. Baratti [b], James L. McGaugh [a,*]

[a] Center for the Neurobiology of Learning and Memory, Department of Neurobiology and Behavior, University of California, Irvine, CA 92697-3800, USA
[b] Laboratorio de Neurofarmacología de los Procesos de Memoria Cátedra de Farmacología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 18 June 2008
Revised 14 July 2008
Accepted 15 July 2008
Available online 3 September 2008

Keywords:
Extinction
Contextual fear conditioning
Basolateral amygdala
Oxotremorine
Memory consolidation

ABSTRACT

Previous studies have reported that drugs affecting neuromodulatory systems within the basolateral amygdala (BLA), including drugs affecting muscarinic cholinergic receptors, modulate the consolidation of many kinds of training, including contextual fear conditioning (CFC). The present experiments investigated the involvement of muscarinic cholinergic influences within the BLA in modulating the consolidation of CFC extinction memory. Male Sprague Dawley rats implanted with unilateral cannula aimed at the BLA were trained on a CFC task, using footshock stimulation, and 24 and 48 h later were given extinction training by replacing them in the apparatus without footshock. Following each extinction session they received intra-BLA infusions of the cholinergic agonist oxotremorine (10 ng). Immediate post-extinction BLA infusions significantly enhanced extinction but infusions administered 180 min after extinction training did not influence extinction. Thus the oxotremorine effects were time-dependent and not attributable to non-specific effects on retention performance. These findings provide evidence that, as previously found with original CFC learning, cholinergic activation within the BLA modulates the consolidation of CFC extinction.

It is well established that activation of the basolateral amygdala (BLA) modulates the consolidation of memory for many kinds of training experiences (McGaugh, 2004). Many studies have reported that, in rats, posttraining intra-BLA infusions of norepinephrine and drugs that activate β-adrenoceptors induce dose- and time-dependent memory enhancement (McGaugh & Roozendaal, 2002). The β-adrenoceptor antagonist propranolol impairs consolidation and blocks the memory enhancement induced by the opioid and GABAergic receptor antagonists naloxone and bicuculline, respectively (Introini-Collison, Nagahara, & McGaugh 1989; McGaugh, Introini-Collison, Cahill, Kim, & Liang, 1992). There is also extensive evidence that systemic or intra-BLA administration of the muscarinic cholinergic agonist oxotremorine enhances memory consolidation (Baratti, Huygens, Miño, Merlo, & Gardella, 1979; Malin & McGaugh, 2006; Power, McIntyre, Litmanovich, & McGaugh, 2003; Vazdarjanova & McGaugh, 1999). As propranolol does not block oxotremorine memory enhancement, cholinergic influences within the BLA appear to act downstream from noradrenergic activation (Dalmaz, Introini-Collison, & McGaugh, 1993; Introini-Collison, Dalmaz, & McGaugh, 1996).

Most of the evidence indicating that posttraining cholinergic activation of the BLA modulates memory consolidation is based on studies in which drugs were administered after initial training on inhibitory avoidance or contextual fear conditioning (Power & McGaugh, 2002; Power, Vazdarjanova, & McGaugh, 2003; Power et al., 2003; Vazdarjanova & McGaugh, 1999). The present experiments investigated whether cholinergic activation of the BLA also enhances memory of extinction of contextual fear conditioning (CFC). Extinction involves the learning that training cues predict a consequence that is different from that of original learning. In extinction of contextual fear conditioning, exposing a rat to a context where footshock had been delivered enables it to learn the altered cue-consequence prediction and decrease its expression of fear. Thus, as extinction is a form of learning it should be susceptible to posttraining modulation.

An early study investigating this issue found that posttraining systemic administration of the GABAergic antagonist picrotoxin enhanced the extinction of fear conditioning (McGaugh, Castellano, & Brioni, 1990). More recent experiments using CFC found that post-extinction intra-BLA infusions of norepinephrine or bicuculline enhanced consolidation of extinction memory (Berlau & McGaugh, 2006). A study using tone-shock conditioning (Ledgerwood, Richardson, & Cranney, 2003) reported that the partial NMDA agonist D-cycloserine administered either systemically or intra-BLA after extinction training enhanced extinction. Schroeder and Packard (2004) found that either systemic or intra-BLA administration of oxotremorine enhanced the extinction of amphetamine-induced conditioned place preference. However, prior

☆ Supported by NIH Grant MH12526 (JLM).
* Corresponding author. Fax: +1 949 824 2952.
E-mail address: JLMCGAUG@UCI.EDU (J.L. McGaugh).

1074-7427/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.nlm.2008.07.012

studies have not investigated whether intra-BLA infusions of cholinergic drugs affect memory of CFC extinction. To address this issue the present experiments investigated the memory-modulating effects of post-extinction intra-BLA infusions of the muscarinic cholinergic agonist oxotremorine.

Male Sprague Dawley rats (275–301 g at the time of surgery) obtained from Charles River Breeding Laboratories (Boston, MA) were housed individually in a light- and temperature-controlled vivarium (22 °C, 12 h light/dark cycle; lights on at 7:00 AM) with food and water available ad libitum. All procedures were in compliance with NIH guidelines and were approved by the Institutional Animal Care and Use Committee of the University of California, Irvine.

One week after arrival the rats were anesthetized with sodium pentobarbital (55 mg/kg, ip) and injected with 0.2 ml of atropine sulfate intraperitoneally to ensure unobstructed respiration. The head of each rat was affixed to a stereotaxic apparatus (Kopf Instruments, Tujunga, CA). After the skull was exposed, a hole was drilled for the placement of a cannula (15 mm, 23-gauge) into the right BLA The unilateral cannula placement was used because of previous findings from our laboratory indicating that drug infusions administered into the right BLA are more effective than left BLA infusions in influencing memory (Berlau & McGaugh, 2006; LaLumiere & McGaugh, 2005). The cannula was lowered at coordinates 2.8 mm posterior to bregma and 5.0 mm right lateral to the midline, just dorsal to the BLA (−6.5 mm from the skull), and fixed in place with dental cement and two jewel screws attached to the skull. A 15-mm-long stylet was inserted in the cannula to prevent clogging. Saline was administered (sc) to prevent dehydration. The rats were placed in a temperature-controlled incubator and subsequently returned to their home cages and allowed to recover for 7 d. They were then handled for 1 min on each of three consecutive days. During the handling the stylets were removed, and 15-mm-long sham needles were inserted in the cannula to habituate the rats to the injection procedures.

Oxotremorine (Sigma, St. Louis, MO) was dissolved in buffered saline, pH 7.4, to a concentration of 50 ng/μl. A fresh drug solution was prepared before each experiment and was kept in a light-proof vial. Infusions of saline or oxotremorine were made through 30-gauge injection needles connected to a 10 μl Hamilton syringe by polyethylene tubing. The needles protrude 2 mm beyond the tip of the cannulae to reach the BLA. A total of 10 ng in a volume of 0.2 μl was infused by an automated syringe pump (Sage Instruments, Boston, MA) over a period of 23 s. The dose (10 ng) was selected on the basis of prior experiments in our laboratory (Malin & McGaugh, 2006; Vazdarjanova & McGaugh, 1999). The injection needles were retained in place for an additional 60 s to allow for diffusion within the BLA. Drug infusions were made in a room adjacent to the behavioral one where the prior handling procedure was performed.

The results were assessed with one-way analysis of variance (ANOVA), using freezing as between-subjects variable. Results were then analyzed by using Fisher's post hoc test for assessing differences between individual groups. Within subject comparisons were used for conditions where subjects were tested twice. *P* values less than 0.05 were considered significant.

For histological determination of the locus of cannulae tips the rats were anesthetized with an overdose of sodium pentobarbital (100 mg/kg, ip) and perfused intracardially with 0.9% saline and then 10% of formaldehyde. Brains were removed and placed in 10% of formaldehyde overnight and were then cryoprotected in a 30% sucrose solution. The brains were sliced in a freezing microtome at 40 μm sections and then stained with cresyl violet. Slides were then examined under a light microscope. Animals whose cannulae tips were located outside of the BLA were excluded from the analyses.

The training apparatus was a trough-shaped alley (91 cm long, 6.4 cm wide at the bottom, 20 cm wide at the top) separated into two compartments by a sliding-door that retracted into the floor. The light compartment was white and illuminated by a 14 W lamp, and the dark compartment was constructed of stainless steel walls and floor. The apparatus was located in a sound-attenuated, darkened room. For the contextual fear conditioning the rats were placed in the dark compartment, with the door to the light compartment closed, and administered four footshocks (1.0 mA, 1.0 s) at 30 s intervals (see Fig. 1A).

On extinction sessions twenty-four and forty-eight h later the rats were placed in the dark compartment for 2 min and freezing was measured. Freezing was assessed as the absence of all movement except respiration (Fanselow, 1984). In the first experiment the rats received either vehicle or oxotremorine infused in the BLA immediately after each extinction session. In the second experiment the infusions were delayed 180 min after the end of each extinction trial.

The third experiment was designed in order to test for possible non-specific effects of oxotremorine administered without extinction training. Four groups of rats received contextual fear conditioning and extinction training sessions 24 and 48 h later as in the experiments above. Two groups received immediate post-extinction intra-BLA vehicle or oxotremorine infusions. The other two groups were not given extinction training but received intra-BLA vehicle or oxotremorine infusions 24 and 48 h after the contextual fear training. Three days after the original learning all groups were tested, as in the first two experiments (see Fig. 2A).

The last experiment used a reinstatement procedure in order to confirm that the behavioral changes induced by extinction training were due to extinction (see Fig. 3A). It is known that a footshock given in the same context used in extinction of footshock-based training will reinstate the extinguished response, providing evidence that the extinguished response was suppressed, not blocked or forgotten (Bouton & Bolles, 1979). For this test we replicated the

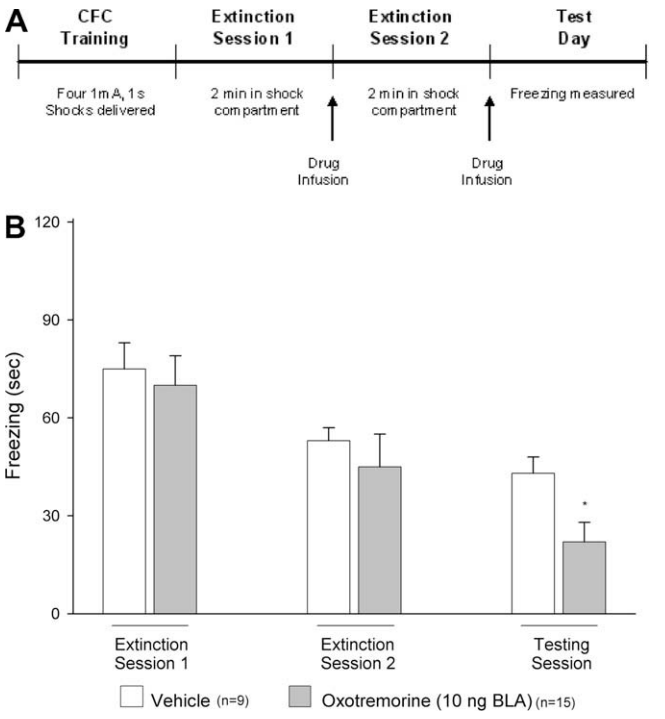

Fig. 1. (A) Behavioral procedure. (B) Freezing behavior in animals with right BLA infusions either of vehicle ($N = 9$) or OXO ($N = 15$) administered immediately after extinction training. $^{*}P < .05$ compared with saline infused animals. Freezing behavior is expressed as mean ± SEM.