Published in final edited form as:

Neurobiol Learn Mem. 2009 January ; 91(1): 93–97. doi:10.1016/j.nlm.2008.07.012.

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

Mariano M. Boccia², Mariano G. Blake², Carlos M. Baratti², and James L. McGaugh¹

¹ Center for the Neurobiology of Learning and Memory and Department of Neurobiology and Behavior, University of California, Irvine, Irvine, CA 92697-3800

² Laboratorio de Neurofarmacología de los Procesos de Memoria, Cátedra de Farmacología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina

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.

Keywords

extinction; contextual fear conditioning; basolateral amygdala; oxotremorine; memory consolidation

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; Vazdarjanova & McGaugh, 1999; Malin & McGaugh, 2006; Power, McIntyre,

Correspondence: James L. McGaugh, EMAIL: E-mail: JLMCGAUG@UCI.EDU.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Litmanovich & McGaugh, 2003). 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 and 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 norpinephrine or bicuculine 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 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 sulphate 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 (LaLumiere, Pizano & McGaugh, 2004; Berlau & McGaugh, 2006). 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/ μ l. 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 μ l 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 μ l was infused by an automated syringe pump (Sage Instruments, Boston, MA) over a period of 23 sec. The dose (10 ng) was selected on the basis of prior experiments in our laboratory (Vazdarjanova & McGaugh, 1999; Malin & McGaugh, 2006). The injection needles were retained in place for an additional 60 sec 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 μ m 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 sec) at 30 sec intervals (See Figure 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 h 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 Figure 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 Figure 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 first experiment but, importantly, one day after the test session, an additional extinction session was given so that both the vehicle and oxotremorine groups reached the same low level of freezing. Twenty-four h later the rats were placed in the dark chamber for 120 sec and one mild shock of 0.4 mA, 1.0 s was delivered 60 sec after they were placed in the chamber. In order to determine the level of freezing produced by this mild footshock an additional group of animals was given only one footshock of 0.4 mA, 1.0 s for CFC training and tested 24 h later. On that test session the rats were placed in the white chamber, with the sliding-door opened, and latencies to step-through to the dark compartment and freezing were measured for 120 sec. If the rats did not cross into the dark chamber a ceiling score was assigned (120 sec).

Figure 1B shows the retention performance of rats that received intra-BLA infusions of the vehicle or oxotremorine immediately after extinction training. The groups did not differ in per cent freezing on the first extinction training session (ANOVA, $P > 0.05$). Oxotremorine enhanced extinction of freezing after two consecutive days of extinction training, as indicated by freezing on the test session ($P < 0.05$, compared with vehicle control group). Rats given intra-BLA infusions of oxotremorine 3 hr after extinction training did not differ from controls in freezing behavior on the retention test ($P > 0.05$, data not shown).

As shown in Figure 2B, the results of the groups given extinction training replicated the findings of the first experiment. Oxotremorine administered after each extinction training session enhanced extinction as assessed by freezing on the final test (vehicle vs oxotremorine groups; $P < 0.01$). In contrast, infusions of oxotremorine 24 and 48 hr after contextual fear conditioning, but without extinction training, did not affect retention test performance (vehicle vs oxotremorine groups; $P > 0.05$).

As shown in Figure 3B, a reinstatement effect was seen in both the vehicle and oxotremorine-treated groups. For both groups, the amount of freezing displayed on the reinstatement test was greater than that on the preceding test session (within subject analysis, $P < 0.01$). Further, the two groups did not differ in amount of freezing on the reinstatement test, a finding that was expected, as they had received an additional extinction session to equate the freezing behavior prior to the reinstatement session. Importantly, the control group given only the mild footshock did not show significant freezing on the test. The freezing displayed by the groups given reinstatement training was significantly greater than that of the reinstatement control group that received only the mild shock ($P < 0.01$ for both comparisons). The vehicle and oxotremorine-treated groups did not differ significantly in latencies to enter the shock compartment on the reinstatement test ($P > 0.05$). The mean entrance latency for each group was 120 sec, the total time allowed for the retention test.

The main finding of the present experiments is that posttraining muscarinic cholinergic activation of the BLA enhances CFC extinction as assessed by freezing behavior in the training context. Further the finding that the effects were time-dependent, that is, that immediate post-extinction intra-BLA infusions of oxotremorine enhanced extinction but infusions administered 180 min after extinction did not, provides strong evidence that the oxotremorine infusion enhanced the consolidation of extinction memory. The additional finding that a mild footshock that was insufficient to induce CFC reinstated the extinguished response provided evidence that the decreased responding induced by extinction training was due to CFC extinction and not to other possible effects of the extinction training or intra-BLA drug infusions.

The results are highly comparable to those of a prior study of the enhancing effects of immediate post-extinction intra-BLA infusions of norepinephrine and bicuculline on CFC extinction (Berlau and McGaugh, 2006). Thus, the findings provide further evidence that neuromodulatory influences within the BLA that are known to enhance original learning (McGaugh, 2004) also enhance extinction learning. Extensive prior evidence indicates that BLA cholinergic influences on consolidation of memory of original training (Vazdarjanova & McGaugh, 1999; Malin & McGaugh, 2006; Power et al., 2003) act downstream from (most likely immediately subsequent to) GABAergic and noradrenergic influences (Dalmaz et al., 1993; Introini-Collison et al., 1996; McGaugh, 2004). Such findings suggested that post-extinction intra-BLA infusions of oxotremorine would induce enhanced extinction comparable to that induced by norepinephrine and bicuculline. The findings provide strong evidence supporting this implication. The findings are also consistent with the evidence that post-extinction intra-BLA infusions of oxotremorine enhanced the extinction of amphetamine-induced conditioned place preference (Schroeder & Packard, 2004). Extensive evidence indicates that modulatory influences within the BLA enhance memory consolidation via activation of efferent brain regions involved in processing different kinds of learning experiences, including object recognition, conditioned taste aversion and spatial learning, as well as inhibitory avoidance and contextual fear conditioning (McGaugh, 2004, McGaugh & Roozendaal, 2002; Malin & McGaugh, 2006; Miranda and McGaugh, 2004; Roozendaal, Okuda, Van der Zee & McGaugh, 2006).

As noted above, our findings are based on assessment of CFC-induced freezing behavior. The use of a maximum latency of 120 sec may have prevented determination of decreases in latencies. In prior studies we have found that lesions or reversible inactivation of the BLA that impair consolidation of original learning as assessed by decreased freezing behavior on 120 sec tests do not always significantly decrease response latencies (Berlau & McGaugh, 2003; Vazdarjanova & McGaugh, 1998).

The procedures used in the present experiments are like those used in studies investigating the “reconsolidation hypothesis:” Each extinction session retrieved memory of the original CFC training. According to the “reconsolidation hypothesis, retrieval of a memory makes the memory trace labile and induces re-consolidation of the trace” (Przybylski & Sara, 1997; Myers & Davis, 2002). Thus, as oxotremorine enhances the consolidation memory of the original learning it should be expected to enhance the reconsolidation of the memory and prevent extinction. Our finding that post-extinction intra-BLA infusions of oxotremorine enhance extinction clearly does not support that implication.

In summary, the present findings indicate that oxotremorine infused post-extinction into the right BLA enhances extinction memory consolidation and, thus, that the neuromodulatory cholinergic system within the BLA works in a similar manner for both fear learning and fear extinction. These findings provide additional evidence that the BLA plays a modulatory role in the consolidation of different kinds of information (McGaugh, 2004).

Acknowledgements

Supported by NIH Grant MH12526 (JLM).

References

- Baratti CN, Huygens P, Miño J, Merlo A, Gardella J. Memory facilitation with posttrial injection of oxotremorine and physostigmine in mice. *Psychopharmacology* 1979;64:85–88. [PubMed: 113837]
- Berlau D, McGaugh JL. Basolateral amygdala lesions do not prevent memory of context-footshock training. *Learning and Memory* 2003;10:495–502. [PubMed: 14657260]

- Berlau DJ, McGaugh JL. Enhancement of extinction memory consolidation: The role of the noradrenergic and GABAergic systems within the basolateral amygdala. *Neurobiology of Learning and Memory* 2006;86:123–132. [PubMed: 16458544]
- Bouton ME, Bolles RC. Role of conditioned contextual stimuli in reinstatement of extinguished fear. *Journal of Experimental Psychology: Animal Behavior Process* 1979;5:368–378.
- Dalmaz C, Introini-Collison IB, McGaugh JL. Noradrenergic and cholinergic interactions in the amygdala and the modulation of memory storage. *Behavioural Brain Research* 1993;58:167–174. [PubMed: 8136043]
- Fanselow MS. What is conditioned fear? *Trends in Neurosciences* 1984;7:460–462.
- Introini-Collison IB, Nagahara AH, McGaugh JL. Memory-enhancement with intra-amygdala posttraining naloxone is blocked by concurrent administration of propranolol. *Brain Research* 1989;476:94–101. [PubMed: 2914216]
- Introini-Collison IB, Dalmaz C, McGaugh JL. Amygdala beta-noradrenergic influences on memory storage involve cholinergic activation. *Neurobiology of Learning and Memory* 1996;65:57–64. [PubMed: 8673407]
- LaLumiere RT, Pizano E, McGaugh JL. Intra-basolateral amygdala infusions of AP-5 impair or enhance retention of inhibitory avoidance depending on training conditions. *Neurobiology of Learning and Memory* 2004;81:60–66. [PubMed: 14670359]
- Ledgerwood L, Richardson R, Cranney J. Effects of D-cycloserine on extinction of conditioned freezing. *Behavioral Neuroscience* 2003;117:341–349. [PubMed: 12708530]
- Malin E, McGaugh JL. Differential involvement of the hippocampus, anterior cingulate cortex and basolateral amygdala in memory for context and footshock. *Proceedings, National Academy of Sciences, USA* 2006;103:1959–1963.
- McGaugh JL. The amygdala modulates the consolidation of memories of emotionally arousing experiences. *Annual Review of Neuroscience* 2004;27:1–28.
- McGaugh JL, Castellano C, Brioni JD. Picrotoxin enhances latent extinction of conditioned fear. *Behavioral Neuroscience* 1990;104:262–265.
- McGaugh JL, Roozendaal B. Role of adrenal stress hormones in forming lasting memories in the brain. *Current Opinion in Neurobiology* 2002;12:205–210. [PubMed: 12015238]
- McGaugh, JL.; Introini-Collison, IB.; Cahill, L.; Kim, M.; Liang, KC. Involvement of the amygdala in neuromodulatory influences on memory storage. In: Aggleton, J., editor. *The Amygdala*. New York: John Wiley and Sons, Inc.; 1992. p. 431–451.
- Miranda MI, McGaugh JL. Enhancement of inhibitory avoidance and conditioned taste aversion memory with insular cortex infusions of 8-Br-cAMP: Involvement of the basolateral amygdala. *Learning Memory* 2004;11:312–317. [PubMed: 15169861]
- Myers KM, Davis M. Systems-level reconsolidation: Re-engagement of the hippocampus with memory reactivation. *Neuron* 2002;36:340–343. [PubMed: 12408838]
- Power AE, McGaugh JL. Cholinergic activation of the basolateral amygdala regulates unlearned freezing behavior in rats. *Behavioural Brain Research* 2002;134:307–315. [PubMed: 12191818]
- Power AE, Vazdarjanova A, McGaugh JL. Muscarinic cholinergic influences in memory consolidation. *Neurobiology of Learning and Memory* 2003;80:178–193. [PubMed: 14521862]
- Power AE, McIntyre CK, Litmanovich A, McGaugh JL. Cholinergic modulation of memory in the basolateral amygdala involves activation of both m1 and m2 receptors. *Behavioral Pharmacology* 2003;14:207–213.
- Przybylski J, Sara SJ. Reconsolidation of memory after its reactivation. *Behavioural Brain Research* 1997;84:241–246. [PubMed: 9079788]
- Roozendaal B, Okuda S, Van der Zee EA, McGaugh JL. Glucocorticoid enhancement of memory requires arousal-induced noradrenergic activation in the basolateral amygdala. *Proceedings, National Academy of Sciences, USA* 2006;101:11454–11458.
- Schroeder JP, Packard MG. Facilitation of memory for extinction of drug-induced conditioned reward: role of amygdala and acetylcholine. *Learning and Memory* 2004;11:641–647. [PubMed: 15466320]
- Vazdarjanova A, McGaugh JL. Basolateral amygdala is not critical for cognitive memory of contextual fear conditioning. *Proceedings, National Academy of Sciences, USA* 1998;95:15003–15007.

Vazdarjanova A, McGaugh JL. Basolateral amygdala is involved in modulating consolidation of memory for classical fear conditioning. *Journal of Neuroscience* 1999;19:6615–6622. [PubMed: 10414989]

Figure 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.

* $p < 0.05$ compared with saline infused animals. Freezing behavior is expressed as mean $\pm$ SEM.

Figure 2.

A: Behavioral Procedure

B: Freezing behavior on the test day, in animals with right BLA infusions either of Vehicle or OXO in rats either given (Ext) or not given (N-Ext) extinction training.

Veh-Ext, N=12; Veh N-Ext, N=9; Oxo-Ext, N=15; Oxo N-Ext, N= 9

** $p < 0.01$ compared with saline infused animals. Freezing behavior is expressed as mean $\pm$ SEM.

Figure 3.

A: Behavioral Procedure

B: Freezing behavior in animals given a reinstatement session. Contextual fear memory was recovered after a mild footshock in animals where original memory was extinguished. This mild footshock *per se* did not induce a high level of freezing.

** $p < 0.01$ compared with the Vehicle ($N=9$) or Oxotremorine ($N=15$) treated group. ## $p < 0.01$ comparing test session with reinstatement session. Freezing behavior is expressed as mean $\pm$ SE.