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Enhancement of extinction memory consolidation: The role of the noradrenergic and GABAergic systems within the basolateral amygdala

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Abstract

Evidence from previous studies indicates that the noradrenergic and GABAergic influences within the basolateral amygdala (BLA) modulate the consolidation of memory for fear conditioning. The present experiments investigated whether the same modulatory influences are involved in regulating the extinction of fear-based learning. To investigate this issue, male Sprague Dawley rats implanted with unilateral or bilateral cannula aimed at the BLA were trained on a contextual fear conditioning (CFC) task and 24 and 48 h later were given extinction training. Immediately following each extinction session they received intra-BLA infusions of the GABAergic antagonist bicuculline (50 ng), the β-adrenocepter antagonist propranolol (500 ng), bicuculline with propranolol, norepinephrine (NE) (0.3, 1.0, and 3.0 μg), the GABAergic agonist muscimol (125 ng), NE with muscimol or a control solution. To investigate the involvement of the dorsal hippocampus (DH) as a possible target of BLA activation during extinction, other animals were given infusions of muscimol (500 ng) via an ipsilateral cannula implanted in the DH. Bilateral BLA infusions of bicuculline significantly enhanced extinction, as did infusions into the right, but not left BLA. Propranolol infused into the right BLA together with bicuculline blocked the bicuculline-induced memory enhancement. Norepinephrine infused into the right BLA also enhanced extinction, and this effect was not blocked by co-infusions of muscimol. Additionally, muscimol infused into the DH did not attenuate the memory enhancing effects of norepinephrine infused into the BLA. These findings provide evidence that, as with original CFC learning, noradrenergic activation within the BLA modulates the consolidation of CFC extinction. The findings also suggest that the BLA influence on extinction is not mediated by an interaction with the dorsal hippocampus.

Keywords: Bicuculline; Norepinephrine; Propranolol; Muscimol; Hippocampus; Contextual fear conditioning

1. Introduction

Contextual fear conditioning (CFC) is induced by pairing a context (conditioned stimulus; CS) with a fear-inducing stimulus (unconditioned stimulus; US), usually footshock. Subsequent elicitation of autonomic and behavioral fear responses, such as freezing, by the CS is typically used as an index of the CS–US association (Blanchard & Blanchard, 1972). CFC responses, like other forms of learned responses, can be extinguished by presenting the CS alone. The new information that the context no longer predicts a footshock results in a decrease in the expression of the conditioned response (CR) (Pavlov, 1927). Extinction shares many common properties with original learning. Both require the formation of CS–US predictions and both require consolidation to achieve stability (McGaugh, 2000).

Considerable evidence indicates that the basolateral nucleus of the amygdala (BLA) plays an important role in modulating the consolidation of fear-based memories, including CFC (Berlau & McGaugh, 2003; Huff, Wright-Hardesty, Higgins, Matus-Amat, & Rudy, 2005; Kim & Jung, 2005; LaLumiere, Buen, & McGaugh, 2003; McGaugh, 2002; Vazdarjanova & McGaugh, 1999; Wilensky, Schafe, & LeDoux, 2000). There is extensive evidence that post-training noradrenergic agonists and antagonists enhance and impair, respectively, the consolidation

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of fear-based memory when infused into the BLA (Ferry, Roozendaal, & McGaugh, 1999; LaLumiere et al., 2003; Power, Thal, & McGaugh, 2002). Findings of studies using in vivo microdialysis and high-performance liquid chromatography (HPLC) provide additional evidence that endogenous NE release in the amygdala plays a role in memory modulation (Galvez, Mesches, & McGaugh, 1996; McIntyre, Hatfield, & McGaugh, 2002; Quirarte, Galvez, Roozendaal, & McGaugh, 1998).

Other neuromodulatory systems, including the GABAergic system, also modulate memory consolidation by regulating NE release within the amygdala. Post-training systemic administration or direct amygdala infusions of GABAergic agonists and antagonists impair and facilitate memory consolidation, respectively (Breen & McGaugh, 1961; Brioni & McGaugh, 1988; Brioni, Nagahara, & McGaugh, 1989; Castellano, Brioni, Nagahara, & McGaugh, 1989). Post-training intra-BLA (Cahill & McGaugh, 1996) or systemic (Introini-Collison, Castellano, & McGaugh, 1994) administration of a β-adrenergic antagonist blocks the memory-modulating effects of systemically administered GABAergic drugs. Such findings support the view that GABAergic influences on memory consolidation act upstream from noradrenergic activation by modulating NE release. Moreover, experiments using in vivo microdialysis have shown that systemic administration of the $GABA_A$ antagonist picrotoxin increases the release of NE in the amygdala, whereas the $GABA_A$ agonist muscimol decreases the release (Hatfield, Spanis, & McGaugh, 1999). In addition, GABAergic antagonists potentiate footshock-induced NE release in the amygdala (Quirarte et al., 1998).

There is extensive evidence that the BLA modulates memory consolidation via its efferent connections to other brain structures (McGaugh, 2000, 2004). The projections between the hippocampus and amygdala play an important and perhaps critical role in the consolidation of contextual fear memories (McIntyre et al., 2005; Roozendaal & McGaugh, 1997). This might be expected, in view of the extensive evidence that the hippocampus is engaged by contextual learning (Eichenbaum, Schoenbaum, Young, & Bunsey, 1996; Hirsh, 1974; Matus-Amat, Higgins, Barrientos, & Rudy, 2004; McNish, Gewirtz, & Davis, 1997; Phillips & LeDoux, 1992; Rudy & Sutherland, 1989) and receives substantial input from the BLA either directly or indirectly via the entorhinal cortex (Alheid, de Olmos, & Beltramino, 1995; Racine, Milgram, & Hafner, 1983; Thomas, Assaf, & Iversen, 1984). Infusions of β-adrenoceptor antagonists into the BLA block the memory modulating effects of drugs infused post-training into the hippocampus (Roozendaal, Nguyen, Power, & McGaugh, 1999).

There is also extensive evidence that the BLA is involved in extinction. Several studies have reported that infusions of drugs into the BLA affect extinction in experiments using tasks other than contextual fear conditioning, such as conditioned taste aversion (Bahar, Samuel, Hazvi, & Dudai, 2003). Infusions of the MAPK inhibitor PD98059 into the BLA impair extinction of fear-potentiated startle (FPS) whereas such infusions administered the hippocampus do not affect FPS extinction (Lu, Walker, & Davis, 2001). Furthermore, intra-amygdala infusions of the NMDA receptor antagonist AP5 impair extinction of FPS, whereas infusions into a control brain region, such as the cerebellum, have no effect (Falls, Miserendino, & Davis, 1992). Administration of D-cycloserine (DCS), a partial NMDA receptor agonist, enhances extinction of FPS when administered either systemically or directly into the amygdala before extinction training (Walker, Ressler, Lu, & Davis, 2002).

In several studies, drugs were administered shortly after extinction training to affect memory consolidation without affecting processes influencing animals' experiences at the time of extinction training. Ledgerwood, Richardson, and Cranney (2003, 2005) reported that intra-amygdala infusions of the partial NMDA agonist DCS administered immediately following an extinction trial enhanced extinction retention. Additionally, an earlier study from our laboratory found that systemic administration of the $GABA_A$ agonist picrotoxin immediately after extinction training enhanced the extinction of auditory fear conditioning (McGaugh, Castellano, & Brioni, 1990). Such findings clearly suggest that GABAergic influences modulate extinction memory consolidation in a similar manner to that of original memory consolidation (Brioni & McGaugh, 1988).

The present study investigated the involvement of the GABAergic and noradrenergic systems within the BLA in the consolidation of extinction memory. The experiments investigated whether, as with initial consolidation of fear-based memory (Brioni et al., 1989; Castellano et al., 1989), the consolidation of extinction involves GABAergic influences in the BLA and whether such effects are also mediated by noradrenergic influences (Introini-Collison et al., 1994). The experiments also examined the interaction between the BLA and the dorsal hippocampus (DH) in the consolidation of contextual fear extinction.

2. Materials and methods

2.1. Subjects

Subjects were 267 male Sprague Dawley rats (Charles River, Wilmington, MA) weighing 225–250 g on arrival. The animals were individually housed in a temperature (22 °C) and light (12:12 h light–dark cycle; lights on at 7 am) controlled vivarium. Rats received food and water ad libitum and acclimated to laboratory conditions for 1 week prior to surgery. The UC Irvine Institutional Animal Care and Use Committee approved all procedures.

2.2. Surgical procedures

2.2.1. Basolateral amygdala

The rats were anesthetized with sodium pentobarbital (50 mg/kg ip.), and received atropine sulfate (0.4 mg/kg ip.) to assist breathing. The rats were placed in a small animal stereotaxic frame (Kopf Instruments, Tujunga, CA) with the nose bar maintained at −3.3 mm relative to the interaural line. Guide cannulae aimed at the basolateral amygdala were implanted (2.8 mm posterior and 5.0 mm lateral to Bregma and 6.5 mm ventral to the skull surface) (Paxinos & Watson, 2004).

Some animals were implanted with bilateral BLA cannulae, whereas others were implanted with unilateral cannulae. In some experiments, animals were implanted with cannulae only in the right BLA. The cannulae were constructed of 23-gauge stainless-steel tubing cut to a length of 15.00 (±0.02) mm. The cannulae were affixed to the skull with dental cement. Insect pins (15-mm long 00 insect dissection pins) inserted into the cannulae to maintain patency were removed only for the infusions. After the surgery, the rats were retained in an incubation chamber until they awoke. They were then returned to their home cages and subsequently checked each day, for several days, to ensure that their wounds healed.

2.2.2. Dorsal hippocampus

The surgical procedures were the same as described above except that in addition to a unilateral guide cannula aimed at the right BLA, a cannula was also implanted in the right dorsal hippocampus (3.3 mm posterior and 1.7 mm lateral to Bregma and 2.7 mm ventral to the skull surface) (Paxinos & Watson, 2004).

2.3. Drug infusions

Bicuculline, norepinephrine (NE), propranolol, and muscimol were obtained from Sigma (St. Louis, MO). All drugs were dissolved in either saline (0.9%) or PBS. Bicuculline was administered at one dose (50 ng). Three doses of NE were used (0.1, 1.0, and 3.0 μg). A single dose of propranolol (500 ng) was used. The dosage of propranolol used is not sufficient to impair memory, but blocks memory enhancement (Quirarte, Roozendaal, & McGaugh, 1997). In the BLA, 125 ng of muscimol was infused. All infusions administered into the BLA were 0.2 μL. To infuse the drug or vehicle, PE-20 polyethylene tubing was connected to a 10-μL Hamilton syringe and a 30-gauge dental needle was attached to the other end of the tubing. The infusion needles extended 2 mm beyond the end of the guide cannula (17 mm for BLA). The Hamilton syringe was driven by an automated syringe pump (Sage Instruments) at the rate of 0.38 μL/min. To perform the infusion, the pins were removed from the cannulae and the infusion needles were inserted. The syringe pump was turned on for 32 s to give an infusion volume of 0.2 μL into the BLA. The infusion needles were then left in place for an additional 35 s to allow the solution to diffuse. The animals were returned to their home cages immediately following the infusions.

The dose of muscimol infused into the DH (500 ng in 0.5 μL of phosphate-buffered saline, PBS) was the same dose previously used to inactivate the DH (Corcoran & Maren, 2004). The infusion procedure was the same as described above except the infusion needles extended 1.5 mm beyond the end of the guide cannula (12.5 mm). The syringe pump was turned on for 37 s to infuse 0.5 μL into the DH. The infusion needles were then left in place for an additional 35 s to allow the solution to diffuse. Immediately following the infusions, the animals were returned to their home cages.

2.4. Behavioral apparatus and procedures

The IA 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 alley was divided into two compartments, a safe compartment (31 cm long) constructed of plastic and illuminated by a tensor lamp and a darkened shock compartment (60 cm long) with walls and floors constructed of stainless steel. A stainless-steel door that retracted into the floor separated the compartments. The floor of the dark compartment was connected to an AC shock generator (Lafayette Instruments) controlled by a timer. The apparatus was located in a sound-attenuated, non-illuminated room.

The rats first received CFC training in the apparatus. They were placed into the dark compartment, with the door to the light compartment closed. After the animals explored the dark compartment for 30 s they received a footshock (1.0 mA, 1.0 s) followed by three additional footshocks at 30 s intervals, for a total of four shocks over 2 min. The animals were then removed from the apparatus. For extinction training 24 h later, the animals were placed into the dark compartment facing the closed door and were allowed to explore the dark compartment for 120 s. Freezing behavior, defined as the cessation of all movement except respiration, was recorded. Animals that displayed less than 12 s (10%) freezing on this day were excluded from the experiment to ensure that all animals included in the analyses had formed the original CS–US association as indicated by their freezing behavior. Immediately following extinction training, the rats received either bilateral BLA, unilateral BLA or ipsilateral BLA and DH infusions of drug or vehicle. After the infusions, the rats returned to their home cage. The extinction training and post-training infusions were repeated 24 h later. On a retention test 24 h after the second extinction session, animals received a retention test, in which the animals were placed into the light compartment facing away from the open door and the latency to enter the dark compartment was recorded. The rats were allowed to explore both compartments for 120 s, and time spent freezing was recorded. Fig. 1 summarizes the behavioral procedures used in this study.

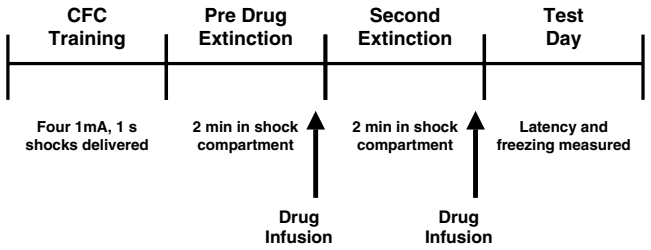

Fig. 1. A summary of the behavioral procedures used in the study. Rats received drug infusions immediately after the first two days of extinction. Freezing was measured on the first two days of extinction, whereas latency and freezing were measured on the test day.

To control for the non-specific effects of the drug infusions, some animals did not receive extinction training. These animals were trained in CFC, but for the following two days, they were placed into a novel cage for 2 min and then given drug infusions. Furthermore, some animals received infusions that were delayed by 3 h. On the final test day, all control rats were treated identically to the other groups.

2.5. Statistics

The results were assessed with one-way analyses of variance (ANOVA) using freezing during retention testing as the between-subjects variable. Results were then analyzed using Fisher's post hoc tests for assessing differences between individual groups. P values of less than .05 were considered significant.

2.6. Histology

After the behavioral tests were completed, the rats were anesthetized with an overdose of sodium pentobarbital (100 mg/kg ip.) and perfused intracardially with 0.9% saline. Brains were then removed and placed in 10% formaldehyde for a minimum of 4 h then cryoprotected in a 25% sucrose solution. Sections of 60 μm were taken with a freezing microtome and stained with thionin. Slides were then examined under a light microscope to determine the site of the cannula tips.

3. Results

3.1. Histology

Fig. 2 shows representative needle tracks in both the BLA and the DH. Animals were excluded from the statistical analysis if the infusions caused excessive damage to

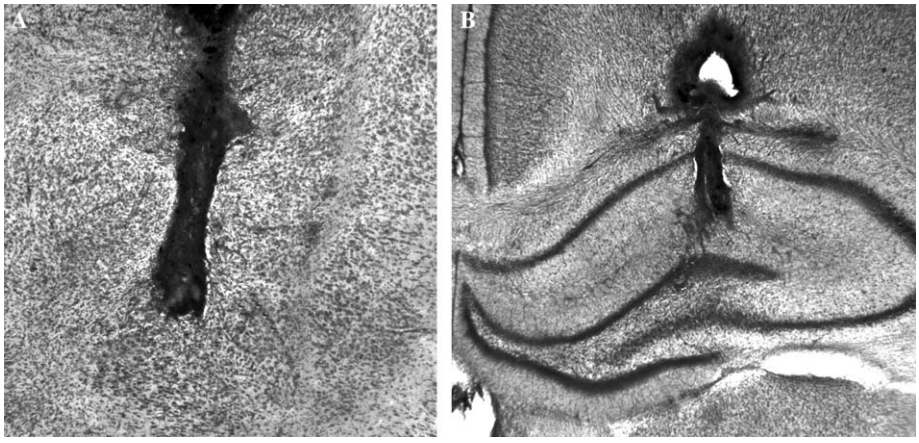

Fig. 2. Photomicrographs of representative needle tracks terminating in the (A) BLA, or (B) DH.

(lesioned) the targeted structure or if the needle tips terminated outside the target structure (191 animals out of 458 total were excluded for these reasons).

3.2. Pre-drug extinction freezing

The groups displayed comparable amounts of freezing behavior on the first extinction session. There were no significant differences between the groups ($P > .05$ in all cases).

3.3. Bicuculline infusions

Fig. 3 shows the freezing behavior of animals given bilateral infusions of bicuculline immediately following the extinction sessions. The bicuculline-infused animals showed significantly less freezing behavior than the saline infused animals on the test day ($P < .05$) using a Fisher's LSD post hoc test. Fig. 4 shows the test day results of the unilateral infusions of bicuculline administered into the BLA. Animals given bicuculline infusions into the right BLA displayed significantly less freezing on the test day than did the saline infused controls ($P < .05$). There was no difference in freezing behavior on the test day between animals given bicuculline into the left BLA and animals given saline ($P > .05$).

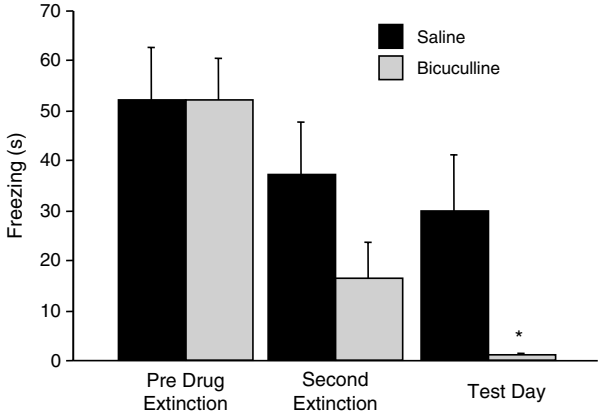

Fig. 3. Freezing behavior in animals with bilateral BLA bicuculline infusions, $^{*}P < .05$ compared with saline infused animals; $n = 9$–10 animals per group.

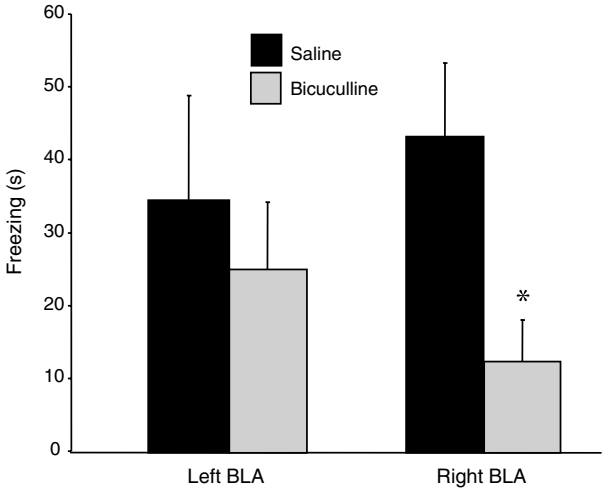

Fig. 4. Freezing behavior on the test day in animals with unilateral infusions of bicuculline into either the right or left BLA. $^{*}P < .05$ compared to saline infused animals; $n = 10$–13 animals per group.

To rule out the effects of bicuculline on processes other than memory consolidation, some animals ($n = 11$) received bicuculline 3 h after extinction training. These animals displayed significantly more freezing than did the animals given immediate unilateral bicuculline infusions ($P < .05$), and their freezing did not differ from that of rats given saline infusions immediately after extinction ($P > .05$). Additionally, in order to control for non-specific effects of the bicuculline in the right BLA, another group of animals ($n = 10$) received bicuculline infusions into the right BLA but was not given extinction training. On the test day, the freezing behavior of these animals was significantly greater than that of the extinguished animals given bicuculline infusions ($P < .05$) and did not differ from that of non-extinguished saline controls ($P > .05$). Fig. 5 shows the results of these animals compared with that of the animals given extinction training.

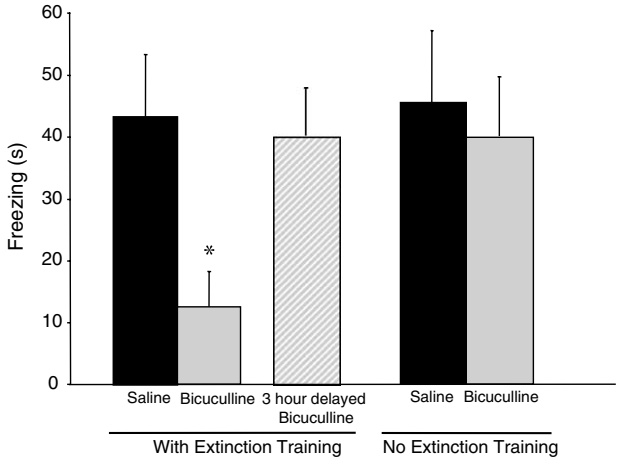

Fig. 5. Freezing behavior on the test day in animals with infusions of bicuculline into the right BLA. $^{*}P < .05$ compared to saline infused animals, animals with delayed infusions and non-extinguished animals receiving bicuculline; $n = 5$–13 animals per group.

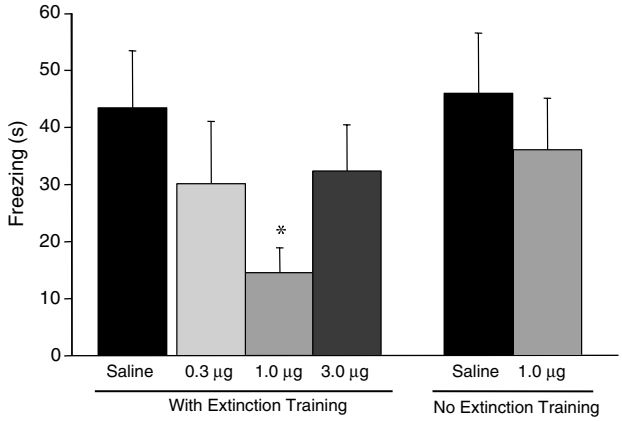

Fig. 7. Freezing behavior on the test day in animals with infusions of norepinephrine into the right BLA. $^{*}P < .05$ compared with saline infused animals (same saline data as Fig. 4) and animals infused with norepinephrine without extinction training; $n = 5$–14 animals per group.

Fig. 6 shows the interaction of propranolol and bicuculline when infused into the right BLA immediately after extinction training. On test day, animals given unilateral infusions of propranolol did not differ in freezing behavior from saline controls ($P > .05$). Additionally, the freezing of rats given infusions of bicuculline and propranolol into the right BLA did not differ from that of the saline controls during the retention test ($P > .05$).

These findings indicate that both bilateral and right side infusions of bicuculline administered into the BLA after extinction training significantly enhanced CFC extinction. Right side bicuculline infusions had no effect if given either without extinction training or 3 h after extinction training. Furthermore, noradrenergic blockade in the BLA prevented the enhancing effects of bicuculline on extinction.

3.4. Norepinephrine infusions

As shown in Fig. 7, animals given post-extinction infusions of NE into the right BLA displayed a dose-dependent enhancement of extinction. On the test day, the animals given the 1.0 µg dose displayed significantly less freezing than that of saline controls ($P < .01$). Doses of 0.3 or 3.0 µg dose did not affect test day freezing behavior ($P > .05$). Again, to control for non-specific effects of the NE into the right BLA, another group of animals ($n = 9$) received 1.0 µg of NE without extinction training, also shown in Fig. 7. On the test day, these animals showed significantly more freezing than that of the extinguished animals given 1.0 µg of NE after extinction ($P < .05$) but their freezing did not differ from that of saline controls not given extinction training ($P > .05$).

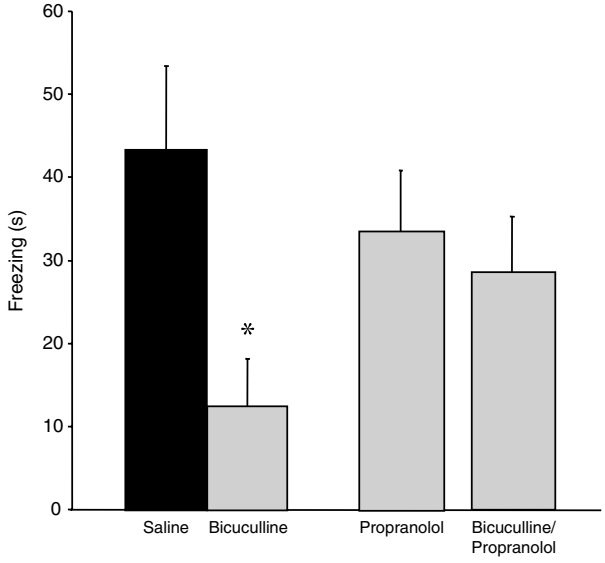

Fig. 6. Freezing behavior on the test day in animals with infusions of bicuculline, propranolol or both into the right BLA. $^{*}P < .05$ compared to saline infused animals (same data as Fig. 4); $n = 11$–17 animals per group.

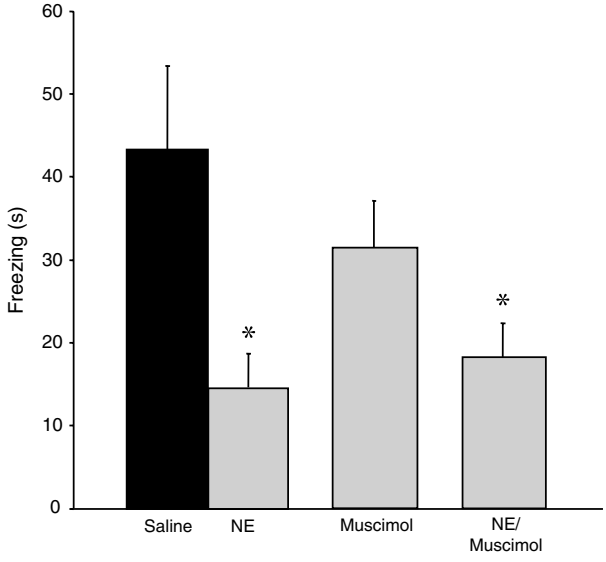

Fig. 8. Freezing behavior on the test day in animals with infusions of norepinephrine, muscimol or both into the right BLA. $^{*}P < .05$ compared to saline infused animals (same saline data as Fig. 4); $n = 13$–16 animals per group.

Fig. 8 shows the interaction of NE and muscimol when infused together into the BLA immediately after extinction training. Animals given unilateral infusions of muscimol did not differ in test-day freezing behavior from saline controls ($P > .05$). However, rats given infusions of both NE and muscimol into the right BLA displayed less test-day freezing than that of saline controls ($P < .05$) and their freezing did not differ from that of rats receiving NE alone ($P > .05$).

These findings indicate that NE infused into the right BLA after extinction training enhanced extinction. The NE infusions did not affect freezing behavior when administered without extinction training. Muscimol infused into the right BLA together with NE did not attenuate the enhancing effect of the NE infusion on extinction.

3.5. Dorsal hippocampus infusions

As shown in Fig. 9, the animals given NE infusions into the BLA and PBS into the DH displayed significantly less freezing on the test day ($P < .01$) in comparison with controls given PBS infusions into both brain areas. Furthermore, animals with NE infusions into the BLA and muscimol infusions into the hippocampus displayed significantly less freezing on the test day ($P < .005$) when compared to controls given PBS into the BLA and muscimol into the hippocampus.

These results provide additional evidence that NE infusions administered into the right BLA enhance extinction. Additionally, reversible inactivation of the ipsilateral DH with muscimol did not block the NE-induced enhancement of extinction, strongly suggesting that dorsal hippocampal functioning is not essential for BLA influences on the extinction of CFC.

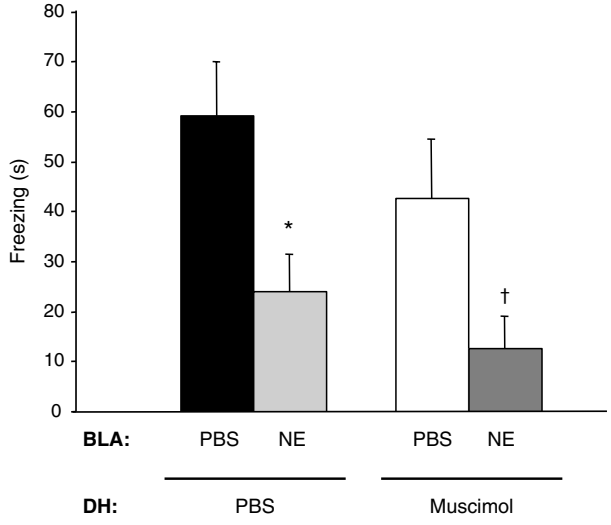

Fig. 9. Freezing behavior on the test day in animals with infusions into the right BLA and right DH $^{*}P < .05$ compared with animals receiving PBS into both the BLA and DH; $^{\dagger}P < .05$ compared with animals receiving muscimol in the DH and PBS into the BLA.

3.6. Latency testing

On the test day, there were no significant differences between any of the groups on retention entrance latencies ($P > .05$ in all cases).

4. Discussion

There are several key findings of these experiments. First, infusions of the GABAergic antagonist bicuculline or NE administered into the BLA immediately after CFC extinction training significantly enhanced extinction as measured by freezing behavior on the test day. BLA infusions administered 3 h after extinction training or infusions given without extinction training did not affect subsequent freezing behavior. Second, post-extinction infusions of the adrenoceptor antagonist propranolol, administered into the BLA together with bicuculline, blocked the enhancement of extinction. Third, the GABAergic agonist muscimol infused together with NE did not block NE-induced enhancement of extinction. Fourth, functional inactivation of the dorsal hippocampus with muscimol infusions did not prevent the enhanced CFC extinction induced by NE infused post-training into the BLA.

As noted, these findings are based on assessment of freezing behavior; the post-training treatments did not significantly affect response (entrance) latencies assessed on the final test trial. Thus, the response latency measure was clearly less sensitive than the freezing measure in assessing CFC extinction. However, the use of a maximum latency of 120 s may have provided a "ceiling" that prevented assessment of decreases in latencies exceeding that "ceiling." That possibility seems likely, as all groups had mean test response latencies at or close to the maximum recorded 120 s. Additionally, previous findings from our laboratory have provided evidence that treatments, such as lesions or reversible inactivation of the BLA, that induce decreases in freezing responses may not significantly decrease rats' latencies to enter a region of an apparatus where they have received footshock (e.g., Berlau & McGaugh, 2003; Vazdarjanova & McGaugh, 1998).

4.1. GABAergic effects in the BLA

The finding that animals given bilateral infusions of bicuculline into the BLA immediately after CFC extinction training displayed significantly less freezing, compared to that of saline controls, supports the view that bicuculline enhanced consolidation of the extinction memory trace. Additionally, as animals given bicuculline infusions either 3 h after extinction or without extinction training did not show a decrease in freezing, it is highly unlikely that the bicuculline had some affect on freezing behavior that was unrelated to extinction. Such findings indicate that the bicuculline influenced the consolidation of extinction memory during a post-training "window" of less than 3 h. These results are similar to those obtained in a previous study investigating the effects of post-

extinction systemic injections of picrotoxin administered to mice (McGaugh et al., 1990).

There is extensive evidence that the GABAergic drugs influence memory consolidation. When administered either systemically or intra-amygdally after inhibitory avoidance training, the antagonists picrotoxin and bicuculline enhance memory and the agonist muscimol impairs memory (Breen & McGaugh, 1961; Brioni & McGaugh, 1988; Brioni et al., 1989; Castellano et al., 1989; Castellano & McGaugh, 1989, 1990). Further, there is evidence that GABAergic drugs affect memory consolidation by modifying NE release within the BLA. Studies using systemically administered drugs administered post-training found that propranolol blocks the memory-enhancing effects of bicuculline and that the β-adrenoceptor agonist clenbuterol blocks the memory-impairing effects of muscimol (Introini-Collison et al., 1994). Additionally, in vivo microdialysis findings indicate that picrotoxin enhances NE release and muscimol decreases NE release (Hatfield et al., 1999).

4.2. Noradrenergic effects in the BLA

Recent research has also implicated the noradrenergic system in extinction, as pre-extinction systemic administration of propranolol has been reported to impair extinction in mice (Cain, Blouin, & Barad, 2004). In the current study, unilateral infusions of NE (1.0 μg) into the BLA enhanced CFC extinction. This dose was also the most effective dose in previous studies investigating the effects of post-training intra-BLA infusions of NE on memory for CFC (Huff et al., 2005; LaLumiere et al., 2003) and inhibitory avoidance (Power et al., 2002). As with bicuculline, the animals that were infused with NE but not given extinction training did not show reduced freezing, indicating that the enhanced extinction induced by NE infusions was due to the extinction training rather than to some other processes that might have affected subsequent freezing.

If CFC extinction memory formation involves the same processes engaged by fear-based memory formation, the memory-enhancing effects of bicuculline on extinction would be expected to require β-adrenergic activation in the BLA. In support of this implication, animals given intra-BLA infusions of bicuculline together with propranolol did not show enhanced extinction. Our finding that muscimol infused together with NE did not block NE-induced enhancement of extinction provides further evidence that NE effects act downstream from those of GABAergic influences (Introini-Collison et al., 1994). Moreover, and importantly, the findings provide clear evidence supporting the hypothesis that the BLA neuromodulatory processes involved in regulating the consolidation of fear-based learning are also involved in regulating the consolidation of CFC extinction.

4.3. Amygdala-hippocampus interactions in extinction

Considerable evidence indicates that the BLA influences the consolidation of fear-based memory, as well as memory for other kinds of training via influences on other brain regions involved in memory (McGaugh, 2002, 2004). Lesions or reversible inactivation of BLA functioning prevent the memory-modulatory effects of drugs administered post-training into several brain regions, including the insular cortex (Miranda & McGaugh, 2004), rostral anterior cingulate cortex (Malin & McGaugh, in press), entorhinal cortex (Roesler, Roozendaal, & McGaugh, 2002) and dorsal hippocampus (Roozendaal & McGaugh, 1997; Roozendaal et al., 1999). There is extensive evidence that the dorsal hippocampus is involved in CFC (Bast, Zhang, & Feldon, 2003; Gale, Anagnostaras, & Fanselow, 2001; Lee & Kesner, 2004; Maren, Aharonov, & Fanselow, 1997; Matus-Amat et al., 2004; McNish et al., 1997; Rudy, Barrientos, & O'Reilly, 2002; Wallenstein & Vago, 2001). Such evidence suggests that BLA influences on CFC extinction may be mediated, at least in part, via interactions of the BLA with the dorsal hippocampus. However, our findings do not support this implication as unilateral inactivation of the dorsal hippocampus with muscimol infusions did not block extinction and did not prevent the enhanced extinction induced by post-training ipsilateral intra-BLA infusions of NE. It is highly unlikely that the BLA influenced extinction by influencing the contralateral hippocampus, as there are no significant contralateral connections between the BLA and the dorsal hippocampus (Pikkarainen, Ronkko, Savander, Insausti, & Pitkanen, 1999).

Several findings suggest that a critical role of the hippocampus in CFC is that of acquiring information about the context associated with the footshock. Infusions of the muscarinic cholinergic agonist oxotremorine into the dorsal hippocampus of rats immediately after they are exposed to a training context enhances the retention of footshock training given subsequently in that context (Malin & McGaugh, in press). The enhancing effects of NE infused into the BLA seen, as indicated above, in many studies are not obtained if rats are given extensive exposure to the context prior to CFC (Huff et al., 2005). However, although the hippocampus may be involved in the extinction of CFC (Chen et al., 2005; Corcoran, Desmond, Frey, & Maren, 2005; Szapiro, Vianna, McGaugh, Medina, & Izquierdo, 2003; Vianna, Igaz, Coitinho, Medina, & Izquierdo, 2003) it may not be required for BLA influences on such extinction. Because contextual information was acquired during the original conditioning, additional information about the context would seem not to be required for learning that the context no longer predicts footshock. This view is consistent with recent evidence indicating that the hippocampus is involved in the formation of a contextual representation, but is not involved in contextual associations (Barrientos, O'Reilly, & Rudy, 2002; Malin & McGaugh, in press). Thus, these findings suggest that BLA modulation of CFC extinction most likely targets a brain structure other than the dorsal hippocampus.

4.4. Extinction and the prefrontal cortex

The several recent studies have reported that extinction of fear-based learning is enhanced by infusions of D-cycloserine,

a partial agonist at the strychnine-insensitive glycine-recognition site on the NMDA receptor, into the amygdala either before (Ledgerwood et al., 2003; Walker et al., 2002) or shortly after (Ledgerwood et al., 2003) extinction training. It might well be that extinction involves changes in circuitry within the BLA and our findings do not exclude that possible interpretation of the findings. Other evidence suggests that extinction of fear-based learning involves interactions of the amygdala with the medial prefrontal cortex (mPFC). In a series of experiments, Quirk and colleagues found that lesions of the mPFC induced prior to extinction training did not affect the acquisition of extinction, but impaired retention of the extinction a day later (Quirk, Russo, Barron, & Lebron, 2000). Moreover, blocking protein synthesis in the mPFC also impairs the recall of extinction (Santini, Ge, Ren, Pena de Ortiz, & Quirk, 2004). Such findings suggest that the mPFC is involved with the long-term storage of extinction, a conclusion further supported by the evidence that neurons in the mPFC (specifically the infralimbic cortex) fire specifically when rats are recalling extinction memories and that electrical stimulation of mPFC reduces freezing to conditioned tones (Milad & Quirk, 2002). Other evidence suggests that the mPFC influences conditioned freezing by exerting inhibitory control over the amygdala (Royer & Paré, 2002) possibly mediated by projections from the mPFC to the intercalated (ITC) cells of the amygdala, which act to inhibit the central nucleus of the amygdala (Quirk, Likhtik, Pelletier, & Paré, 2003).

4.5. Laterality effects

Our findings suggested a larger role of the right BLA than the left, as the infusions of bicuculline were only effective when administered to the animals' right BLA. Previous studies have found that infusions of drugs into the right amygdala alone have effects similar to bilateral infusions (Coleman-Mesches & McGaugh, 1995a, 1995b, 1995c; LaLumiere & McGaugh, 2005). Additionally, human imaging studies have found that activation of the right amygdala tends to correlate with fear learning (Cahill et al., 2001; Canli, Desmond, Zhao, & Gabrieli, 2002). This difference in activation may be a sex-related difference, as males tend to have greater activation on the right side whereas females usually have greater left amygdala activation (Cahill, Uncapher, Kilpatrick, Alkire, & Turner, 2004).

In summary, the present findings indicate that manipulations of the BLA can enhance extinction memory consolidation and that the neuromodulatory system within the BLA works in a similar manner for both fear learning and fear extinction. These findings thus provide additional evidence that the BLA plays a modulatory role in the consolidation of different kinds of information (McGaugh, 2004).

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