Modulation of memory consolidation by the basolateral amygdala or nucleus accumbens shell requires concurrent dopamine receptor activation in both brain regions

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Previous findings indicate that the basolateral amygdala (BLA) and the nucleus accumbens (NAc) interact in influencing memory consolidation. The current study investigated whether this interaction requires concurrent dopamine (DA) receptor activation in both brain regions. Unilateral, right-side cannulae were implanted into the BLA and the ipsilateral NAc shell or core in male Sprague-Dawley rats (∼300 g). One week later, the rats were trained on an inhibitory avoidance (IA) task and, 48 h later, they were tested for retention. Drugs were infused into the BLA and NAc shell or core immediately after training. Post-training intra-BLA infusions of DA enhanced retention, as assessed by latencies to enter the shock compartment on the retention test. Infusions of the general DA receptor antagonist cis-Flupenthixol (Flu) into the NAc shell (but not the core) blocked the memory enhancement induced by the BLA infusions of DA. In the reverse experiment, post-training intra-NAc shell infusions of DA enhanced retention and Flu infusions into the BLA blocked the enhancement. These findings indicate that BLA modulation of memory consolidation requires concurrent DA receptor activation in the NAc shell but not the core. Similarly, NAc shell modulation of memory consolidation requires concurrent DA receptor activation in the BLA. Together with previous findings, these results suggest that the dopaminergic innervation of the BLA and NAc shell is critically involved in the modulation of memory consolidation.

Considerable evidence suggests that the basolateral amygdala (BLA) is involved in modulating the consolidation of memory for training in a variety of tasks, including inhibitory avoidance (IA), contextual fear conditioning, spatial and cued water maze, and conditioned taste aversion (Packard et al. 1994; Hatfield and McGaugh 1999; LaLumiere et al. 2003, 2004; Miranda et al. 2003). Several neurotransmitter systems within the BLA, including noradrenaline (NA), acetylcholine (ACh), and dopamine (DA), are known to influence memory consolidation (LaLumiere et al. 2003, 2004; Power et al. 2003).

Extensive evidence indicates the BLA influences memory consolidation through its effects on several forebrain structures, including the hippocampus, the caudate nucleus, the insular cortex, and the nucleus accumbens (NAc) (Packard et al. 1994, 1996; Roozendaal et al. 1999, 2001; Miranda and McGaugh 2004). The BLA has a direct, ipsilateral glutamatergic projection to the NAc through the stria terminalis (Kelley et al. 1982; Christie et al. 1987; Robinson and Beart 1988). The NAc appears to play a critical role in the BLA-mediated memory modulation, as lesions of the NAc or the stria terminals prevent the memory-enhancing effects of glucocorticoid agonist infusions administered into the BLA after IA training (Roozendaal et al. 2001). Although the BLA projects to both the NAc shell and core, evidence suggests a greater role for the DA system in the shell during emotionally influenced memory consolidation, as DA release during fear conditioning is greater in the shell than in the core (Peze et al. 2002).

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Results

Histology

Infusions into the rats were conducted via needles tracking into the BLA or the NAc shell or core. Figure 1 shows diagrams of the

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selected rats included in the final analysis (20 with shell infusions and 20 with core infusions). Figure 1G shows the corresponding needle infusion termination sites in the NAc shell or core.

**Experiment 1**

The retention latencies of rats given immediate post-training intra-BLA infusions of DA and intra-NAc shell infusions of the general DA receptor antagonist *cis*-Flupenthixol (Flu) are shown in Figure 2A. A two-way analysis of variance (ANOVA) revealed a significant main effect of the intra-NAc shell infusions ($F_{(2,44)}=15.016$, $p<0.001$), a marginally insignificant main effect of the intra-BLA infusions ($F_{(2,44)}=2.982$, $p<0.065$), and a significant interaction between the intra-NAc shell infusions and the intra-BLA infusions ($F_{(2,44)}=3.647$, $p<0.05$). The latencies of rats given intra-NAc shell infusions of vehicle and intra-BLA infusions of either DA dose (3.0 µg or 10.0 µg) were significantly higher than those of rats given vehicle infusions into the BLA and NAc shell, as well as those of rats given intra-NAc shell infusions of Flu and the respective intra-BLA infusions of DA (3.0 µg or 10.0 µg) ($p<0.01$).

The retention latencies of rats given immediate post-training intra-BLA infusions of DA and intra-NAc core infusions of Flu are shown in Figure 2B. A two-way ANOVA revealed a significant main effect of the intra-BLA infusions ($F_{(2,44)}=3.584$, $p<0.05$). The main effect of the intra-NAc core infusions and the interaction between the intra-BLA and intra-NAc core infusions were not significant ($p>0.5$). The latencies of rats given intra-NAc core infusions of vehicle and intra-BLA infusions of DA (3.0 µg) were significantly higher than those of rats given vehicle infusions into the BLA and NAc core ($p<0.05$) but were not significantly different from those of rats given intra-NAc core infusions of Flu and intra-BLA infusions of DA (3.0 µg). The latencies of rats given intra-NAc core infusions of Flu and intra-BLA infusions of DA (3.0 µg) were higher than those of rats given intra-core infusions of Flu and intra-BLA infusions of vehicle, although this effect was marginally insignificant ($p<0.085$).

**Experiment 2**

The retention latencies of rats given immediate post-training intra-BLA infusions of Flu and intra-NAc shell infusions of DA are shown in Figure 3. A two-way ANOVA revealed a significant main effect of the intra-BLA infusions ($F_{(2,49)}=5.602$, $p<0.05$), a marginally insignificant main effect of the intra-NAc shell infu-
Figure 2. (A) Retention of rats given either vehicle or DA (3 µg or 10 µg) into the BLA and either vehicle or Flu (10 µg) into the NAc shell immediately after training. Mean latencies, in sec, (± SEM) to enter the shock compartment during the retention test. Groups (from left to right): Vehicle-vehicle (white bar, n = 10); Vehicle-DA (3 µg) (hatched bar, n = 9); vehicle-vehicle (10 µg), (black bar, n = 6); Flu-vehicle (black bar, n = 6); Flu-DA (3 µg (hatched bar, n = 9); and Flu-DA (10 µg) (black bar, n = 16). Bars represent mean latencies, in seconds, (± SEM) to enter the shock compartment during the retention test. *p < 0.001 compared with vehicle-vehicle and flu-DA (3 µg). #p < 0.01 compared with vehicle-vehicle and Flu-DA (10 µg). (B) Retention of rats given either vehicle or DA (3 µg or 10 µg) into the BLA and either vehicle or Flu (10 µg) into the NAc core immediately after training. Mean latencies, in sec, (± SEM) to enter the shock compartment during the retention test. Groups (from left to right): Vehicle-vehicle (white bar, n = 12), Vehicle-DA (3 µg) (hatched bar, n = 13), vehicle-vehicle (10 µg), (black bar, n = 14), Flu-vehicle (white bar, n = 16), Flu-DA (3 µg) (hatched bar, n = 11), and Flu-DA (10 µg) (black bar, n = 11). Bars represent mean latencies, in sec, (± SEM) to enter the shock compartment during the retention test. *p < 0.05 compared with vehicle-vehicle. #p < 0.005 compared with Flu-vehicle.

Discussion

Our findings indicate that modulation of memory consolidation induced by DA infusions into either the BLA or NAC requires concurrent activation of DA receptors in both brain regions. The results of Experiment 1 indicate that post-training intra-NAc infusions of DA enhanced retention of IA training and that concurrent intra-NAc shell infusions of Flu blocked this memory enhancement. However, concurrent intra-NAc core infusions of Flu did not affect the BLA-mediated memory enhancement. It is not clear why the latencies of control animals given intra-NAc core infusions were higher than those of control animals given intra-NAc shell infusions; however, the higher overall latencies in the NAC core experiment may have produced a ceiling effect, leading to the marginally insignificant difference between the group receiving core infusions of Flu and BLA infusions of 3.0 µg DA and its respective control group. Thus, Experiment 2 used a slightly lower footshock. The results of Experiment 2 indicate that post-training intra-NAc shell infusions of DA enhanced retention of IA training and that concurrent intra-BLA infusions of Flu blocked this memory enhancement. These findings strongly suggest that activation of DA receptors within both the NAC shell and the BLA is critical for modulating the consolidation of memory for IA training. Although infusions of DA into the BLA and NAC shell were used in these experiments to enhance memory, we believe that our findings are not specific to enhancement induced by DA infusions. Memory enhancement induced by intra-BLA or intra-NAc shell infusions of drugs affecting other neurotransmitter systems would also most likely be blocked by Flu infusions into the other structure.

Our findings are consistent with other evidence suggesting an interaction between the NAC and BLA during memory consolidation. The BLA provides a glutamatergic innervation of the NAC via the stria terminals (Kelley et al. 1982; Christie et al. 1987; Robinson and Bear 1988). Lesions of the stria terminalis prevent the memory enhancement induced by post-training intra-BLA infusions of drugs affecting several neuromodulatory systems (Liang et al. 1990; Roozendaal et al. 2001; McGaugh 2004). Moreover, lesions of the NAC block the memory enhancement induced by post-training intra-BLA infusions of glucocorticoid agonists (Roozendaal et al. 2001). Unilateral, contralateral lesions of the NAC and BLA, but not ipsilateral lesions, prevent the enhancement of memory induced by systemic injections of a glucocorticoid agonist, suggesting that the BLA-NAC pathway is critically involved in the modulation of memory consolidation by stress hormones (Setlow et al. 2000).

Our findings strongly indicate that the activation of DA receptors within the NAC shell, but not the core, is a critical component in the modulation of memory consolidation and that the BLA influences consolidation, at least in part, through effects on DA release within the NAC shell. The NAC receives a significant DA innervation from the VTA (Fallon and Loughlin 1995; Heimer et al. 1995). However, electrical stimulation of the BLA increases DA release in the NAc, even when the VTA or the medial prefrontal cortex is inactivated (Floresco et al. 1998; Howland et al. 2002). This effect appears to be mediated by the glutamatergic projection by the BLA, as glutamatergic antagonists infused into the NAC block the BLA stimulation-induced increase in DA release in the NAc (Floresco et al. 1998).
Although the results for the core-infused groups were not as clear as those for the shell-infused groups in Experiment 1, they suggest that intra-core infusions of Flu do not prevent the memory enhancement induced by DA infusions into the BLA. This finding is consistent with other evidence suggesting a larger increase in DA release in the shell than in the core during fear conditioning training (Pezze et al. 2002) and following a mildly stressful stimulus (Barrot et al. 2000). In addition, it appears that the transient increase in DA release following the mildly stressful stimulus found in the shell, but not the core, depends on circulating corticosterone (Barrot et al. 2000), a stress hormone known to enhance memory consolidation (Roozendaal and McGaugh 1996; Hui et al. 2004). However, the NAc core may still play a role during memory consolidation, as post-training intra-core infusions of a D2 antagonist impair memory for spatial water maze training (Setlow and McGaugh 1999).

The findings of Experiment 2 are particularly significant, as they are the first to demonstrate memory enhancement for training in an aversive task by activating DA receptors in the NAc. Previous studies using aversive learning found that post-training intra-NAc infusions of a glucocorticoid agonist enhance retention (Roozendaal 2000) and that post-training intra-NAc infusions of a DA receptor antagonist impair retention (Setlow and McGaugh 1998, 1999), but they did not examine activation of DA receptors. The current findings are also important because the role of the DA system in the NAc in the learning of an aversive task has been debated (Salamone 1994; Pezze and Feldon 2004; Ungless 2004). Evidence suggests that activation of the dopaminergic system in the NAc induces conditioned place preference (Carr and White 1983) and that rewarding stimuli increases DA release in the NAc (Hernandez and Hoebel 1988). Based on such findings, it has been suggested that DA in the NAc is critically involved in mediating reward (Wise and Rompre 1989) and that, as a consequence, activation of DA receptors in the NAc should impair acquisition/consolidation of training in an aversive task (Schwienbacher et al. 2005). However, the present findings indicate that activation of DA receptors in the NAc enhances memory consolidation for training in an aversive task (IA) and that blockade of those receptors prevents the memory enhancement induced by DA infusions into the BLA.

The NAc, like the BLA, appears to influence memory consolidation through its effects on downstream structures. The NAc projects to three structures: the VTA, the ventral pallidum (VP), and the nucleus basalis magnocellularis (NBM) (Zahm and Heimer 1990; Heimer et al. 1991, 1995; Zaborsky et al. 1991). As the NAc is composed primarily of GABAergic neurons that serve as the projection neurons (Meredith et al. 1992), the NAc retains inhibitory control over the activity of these three structures. Through its projection to the VTA, the NAc regulates the activity of the dopaminergic input to most of the forebrain, except for the dorsal striatum, and may influence the memory consolidation via this input to other structures. The VP projects to the medial dorsal thalamus (Kalivas et al. 1999), which projects to prefrontal cortical regions, which then project back to striatal regions. The NAc, therefore, may regulate the activity of this striatal-pallidal-thalamic-cortical loop and the role these areas play during memory consolidation. Through its projection to the NBM, the NAc influences the activity of the basal forebrain cholinergic neurons, which have widespread projections throughout the cortex and to the BLA as well (Woolf 1991) and are known to influence memory consolidation (Power and McGaugh 2002; Power et al. 2002).

Although all three target structures of the NAc provide possible mechanisms for the influence of the NAc on memory consolidation, recent findings suggest that the NBM cholinergic projections may be critically important for the downstream effect of DA receptor activation in the NAc. Specifically, it appears that DA receptor activation in the NAc modulates NBM cholinergic neuron activity and the release of ACh in the cortex (Moore et al. 1999; Neigh et al. 2004). The findings of the present experiments, together with those of Neigh et al. (2004) and Moore et al. (1999), suggest that the BLA influences memory consolidation, at least in part, by regulating the release of DA in the NAc, which, in turn, influences NBM activity and subsequent release of ACh in the cortex.

The finding that concurrent intra-BLA infusions of Flu block the memory enhancement induced by the DA infusions into the shell is not as easily understood, as the NAc does not project to the BLA. We suggest that the BLA and the NAc shell may play critical roles in a recurrent loop that influences memory consolidation. Increased BLA activity, following emotional arousal, increases DA levels in the NAc, which increase NBM cholinergic neuron activity and VTA dopaminergic neuron activity. Increased activity in these structures will increase ACh and DA release in the BLA. Therefore, this BLA-NAc-NBM/VTA-BLA loop could act to maintain increased activity in the BLA, increased DA levels in the NAc, and increased activity of the NBM-cortical projections. Such a system would enable emotional arousal, via this loop, to influence the consolidation process for a period after the initial learning event through the effects of ACh and DA on cortical plasticity.

Materials and Methods

Subjects

Male Sprague-Dawley rats (approximately 300 grams at the time of surgery, Charles River, Wilmington, MA; n = 256) were used in this study. They were housed individually, maintained in a temperature-controlled environment (22°C) on a 12-h light/12-h dark cycle (lights on at 07:00 h) with food and water ad libitum, and given 7–8 days to acclimatize to the vivarium before undergoing surgery. Behavioral procedures began 7–9 days after surgeries. All methods used complied with NIH guidelines for care of laboratory animals and were approved by the UC Irvine Institutional Animal Care and Use Committee.

Surgery

The rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and given atropine sulfate (0.1 mg, i.p.) to prevent respiratory congestion as well as 3.0 mL of saline (s.c.) to prevent dehydration during surgery. Supplemental doses of sodium pentobarbital were given as needed during surgery. The rats were then placed in a small animal stereotaxic instrument (Kopf Instruments, Tujunga, CA). Two surgical screws were implanted into the skull as anchors and guide cannulae were implanted unilaterally in the right hemisphere, aimed at the BLA (2.8 mm posterior and 5.0 mm lateral to Bregma and 6.5 mm ventral to the skull surface) and at the NAc shell (1.7 mm anterior and 0.9 mm lateral to Bregma and 5.8 mm ventral to the skull surface) or core (1.7 mm anterior and 1.8 mm lateral to Bregma and 5.5 mm ventral to the skull surface) (Paxinos and Watson 1997). The nose bar was maintained at ~3.5 mm relative to the interaural line. The guide cannulae were constructed of 23-gauge stainless steel tubing cut to a length of 15.00 (±0.02) mm. The cannulae and the screws were affixed to the skull with dental cement. Insect pins (15-mm long 00 insect dissection pins) were inserted into the cannulae to maintain patency and were removed only for the infusions. After the surgery, the rats were retained in an incubation chamber until they recovered from the anesthesia. They were then returned to their home cages and checked following surgery to ensure that their wounds healed.

Behavioral procedures

The rats were trained on a step-through IA task. The IA apparatus was a 91-cm-long, 20-cm-deep trough-shaped alley. 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) constructed of stainless steel. The compartments were separated by a stainless steel door that retracted into the floor. The dark compartment’s plates were connected to an AC shock generator (Lafayette Instruments, Lafayette, IN) controlled by a timer. Each rat was handled 1 min per day for three days prior to the start of training. All training and testing occurred between 10:00 a.m. and 4:00 p.m. Immediately after the training and testing of each animal, the apparatus was cleaned with a 20% ethanol solution. On the training day, each rat was placed into the safe compartment, with the door retracted below the floor, and permitted to explore the apparatus freely. After the rat entered the dark compartment, the door was raised to prevent the rat from re-entering the safe compartment. When the rat reached the end of the dark compartment, it received a single inescapable footshock (either 0.45 or 0.5 mA). After the rat was removed from the apparatus, the rat received its appropriate intra-BLA and intra-NAc drug infusions and was replaced in its home cage. Retention was tested 48 h later. Each animal was placed into the safe compartment and permitted to explore the apparatus freely. The animal’s initial latency to step into the dark compartment with all four paws was measured in seconds with a maximum latency of 600 sec.

Drugs and drug infusion procedures

DA and Flu were obtained from Sigma (St. Louis, MO). All drugs were dissolved in saline (0.9%). Two doses of DA were used (3.0 and 10.0 µg) for the intra-NAc infusion and two doses of DA were used for the intra-NAc infusions (4.5 and 15.0 µg), based on previous findings suggesting their efficacy in enhancing memory. A single dose of Flu was used (10.0 µg) for the intra-BLA and intra-NAc infusions. Right-side unilateral, rather than bilateral, infusions were used because recent findings indicate that post-training DA infusions into the left BLA do not affect retention whereas similar infusions into the right BLA enhance retention to levels comparable to those found with bilateral infusions (R.T. LaLumiere and J.L. McGaugh, in prep.). To infuse the drug or vehicle into the BLA or NAc, PE-20 polyethylene tubing was connected to a 10-µL Hamilton syringe and a 30-gauge dental needle was cemented to the other end of the tubing. The infusion needle was then bent to a length of 17 mm so that it extended 2 mm beyond the end of the guide cannula into the BLA or NAc. The tubing was first filled with distilled water. A small air bubble was then pulled in and the drug or vehicle was then pulled in. The Hamilton syringe was driven by an automated syringe pump (Sage Instruments, Boxborough, MA) 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 sec to give an infusion volume of 0.2 µL into the BLA or for 48 sec to give an infusion volume of 0.3 µL into the NAc. The needles were then left in place for an additional 35 sec to allow the solution to diffuse. Immediately following the infusions, the animals were returned to their home cages.

Experiment 1

Rats were trained on the IA task (footshock: 0.5 mA, 1 sec) and given immediate post-training intra-BLA infusions of DA or vehicle (saline) and intra-NAc infusions of Flu or vehicle (saline). To determine whether DA receptor activation in the NAc shell was necessary for memory modulation by the BLA, some rats received infusions of Flu or vehicle into the NAc shell. To determine whether DA receptor activation in the NAc core was necessary for memory modulation by the BLA, other rats received infusions of Flu or vehicle into the NAc core.

Experiment 2

As the findings from Experiment 1 suggested an important role for DA receptors in the NAc shell during memory consolidation, Experiment 2 investigated whether memory modulation by the NAc shell required DA receptor activation in the BLA. Rats were trained on the IA task (footshock: 0.45 mA, 1 sec). A lower footshock was used to reduce the likelihood of a ceiling effect, as occurred during the second part of Experiment 1. Rats received immediate post-training intra-NAc shell infusions of DA or vehicle (saline) and intra-BLA infusions of Flu or vehicle (saline).

Histology

The animals were sacrificed with an overdose of sodium pentobarbital (100 mg/kg) and perfused through the heart with physiological saline (0.9% NaCl). The brains were removed and stored in formaldehyde (4%) for a minimum of 24 h. At least 48 h before the brains were sectioned, the brains were transferred to a 25% sucrose solution. The brains were sectioned (40 µm) on a freezing microtome and mounted onto gelatin-subbed slides. The sections were then stained with thionin. The location of the infusion needles was determined by examining the sections under a microscope and using a rat brain atlas ( Paxinos and Watson 1997). To be included in the final analysis, animals had to have needle tips located in the BLA and no lesions around the needle tips. In addition, animals had to have needle tips located in the NAc shell or core (and only if that was the intended aimed for) and no lesions around the needle tips. Any animals that had needle tips located in the border region between the shell and core were excluded because of the possibility of infusions spreading through both structures.

Statistics

The retention latencies for Experiment 1 and 2 were analyzed with two-way ANOVA, with the BLA infusions and the NAc infusions being the between-subjects variables. Fisher’s post hoc tests were performed to determine the source of detected significances. p-values of less than 0.05 were considered significant. All measures are expressed as mean ± S.E.M. The number of animals in each group is indicated in the figure legends.

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