**Brief Communication**

**Context pre-exposure obscures amygdala modulation of contextual-fear conditioning**

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We report that post-training inactivation of basolateral amygdala region (BLA) with muscimol impaired memory for contextual-fear conditioning (as measured by freezing) and intra-BLA norepinephrine enhanced this memory. However, pre-exposure to the context eliminated both of these effects. These findings provide a likely explanation of why an earlier study failed to observe that the BLA modulates contextual fear conditioning—they pre-exposed their rats to the context. These results also suggest that the amygdala modulates the storage of the context fear memory and may do so by influencing the storage of the representation of the context in which the shock occurred.

There is considerable evidence that the amygdala modulates memory for contextual fear conditioning (Passani et al. 2001; Cangioli et al. 2002; LaLumiere et al. 2003). The findings of Wilensky et al. (2000), however, contrast with this literature. They examined the modulating role of the amygdala on contextual fear conditioning by inactivating neurons in the basolateral (BLA) region with muscimol immediately following conditioning. They found no evidence that the BLA modulates the consolidation of the contextual fear memory. Our experiments are directed at resolving this discrepancy.

Contextual fear conditioning in the intact animal is thought to depend, in part, on a representation of the context that requires the hippocampus (Maren et al. 1997; Fanselow 1999, 2000; Rudy and O’Reilly 2001; Rudy et al. 2004). We recently reported that inactivating the BLA region either before or after exposure to a context impairs the rat’s memory for that context (Huff and Rudy 2004). Based on these results, one might expect that inactivating the amygdala would also impair storage of the contextual fear memory.

It is important to note, however, that Wilensky et al. (2000) pre-exposed their rats to the context for 10 min the day before contextual fear conditioning. Given Huff and Rudy’s (2004) findings, it is reasonable to suppose that the amygdala modulates storage of the contextual fear memory by influencing the hippocampus-dependent context memory component. If this hypothesis is correct, then it is possible that Wilensky et al. (2000) inadvertently obscured the modulation role of the amygdala in contextual fear conditioning because their pre-exposed rats already had acquired a strong representation of the context (Huff and Rudy 2004).

To determine the role context pre-exposure may have played in the Wilensky et al. (2000) experiment, 1 d prior to contextual fear conditioning, rats were pre-exposed to either the conditioning context, A, for 8 min or a different context, B. The following day, rats were conditioned in context A and then muscimol or vehicle was injected bilaterally into BLA. Muscimol is a potent GABA receptor agonist that potentiates inhibitory synaptic transmission in neurons and has been used by our laboratory and several others to inactivate the amygdala (Martin 1991; Coleman-Mesches and McGaugh 1995; Maren et al. 2001; Edeline et al. 2002; Huff and Rudy 2004). If context pre-exposure obscures the modulating role of the amygdala, then muscimol should have no influence on contextual fear conditioning displayed by rats pre-exposed to context A, but it should impair the contextual fear conditioning displayed by rats pre-exposed to context B.

As shown in Figure 1, rats that were pre-exposed to Context A and treated with muscimol (n = 6) after fear conditioning did not differ from their vehicle-injected counterparts (n = 7) who were also pre-exposed to Context A. However, rats that were exposed to Context B and infused with muscimol (n = 5) after contextual fear conditioning did freeze significantly less than their vehicle (n = 5)-infused counterparts. A two-factor ANOVA (Context PreExposure × Drug Treatment) revealed a significant main effect of pre-exposure context, F(1,19) = 5.72, P < 0.03, and a significant main effect of drug treatment, F(1,19) = 10.08, P < 0.01. There was a significant interaction of Context Pre-Exposure × Drug, F(1,19) = 7.28, P < 0.02. Student-Newman-Keuls post-hoc analysis revealed that rats pre-exposed to Context B and infused with intra-amygdala muscimol after fear conditioning displayed less freezing than their vehicle controls (P < 0.05). We replicated Wilensky et al.’s (2000) finding that muscimol had no effect on contextual fear in rats pre-exposed to the conditioning context. However, we also found that muscimol did impair contextual fear conditioning in rats not pre-exposed to the conditioning context. The latter finding suggests that the amygdala modulates memory storage of a single trial of contextual fear conditioning.

Our next experiments were designed (1) to enhance contextual fear conditioning by facilitating the modulatory function of the amygdala and (2) to determine whether this effect can be blocked by context pre-exposure. Previous reports demonstrate that intra-amygdala administration of norepinephrine or β-adrenergic receptor agonists enhance passive avoidance, escape latency, and freezing behavior in a dose-dependent and time-dependent manner (Gallagher et al. 1977; Liang et al. 1986; Ferry and McGaugh 1999; LaLumiere et al. 2003). Given this literature, we expected that infusion of norepinephrine (NE) into the BLA after contextual fear conditioning would enhance memory in a dose-dependent fashion.

In Experiment 2, we infused three doses of norepinephrine into the amygdala immediately after contextual fear conditioning. In order to insure that we could detect a facilitation effect, we reduced the shock intensity to 0.5 mA (see below). In this
of norepinephrine. The conditioning parameters were the same as in Experiment 2. Rats were pre-exposed to either context A or context B. The following day all rats were conditioned in context A. Immediately after conditioning, rats received either a vehicle or norepinephrine (1.0 µg/µL) infusion into the amygdala. Rats were tested in context A ∼24 h later.

Intra-BLA norepinephrine enhanced freezing in rats that were pre-exposed to context B relative to vehicle-infused rats exposed to context B (NE, n = 8, vehicle, n = 8), but this effect was not observed in rats that were pre-exposed to context A, the conditioning context (NE, n = 13, vehicle, n = 12) (Fig. 3). A two-factor ANOVA revealed a significant interaction of Context Pre-Exposure × Drug, \( F_{1,37} = 4.87, P < 0.05 \). Student-Newman-Keuls post-hoc analysis revealed that rats exposed to context B and infused with NE after fear conditioning displayed significantly more freezing than their vehicle-treated counterparts (\( P < 0.05 \)).

A post-conditioning injection of muscimol into the BLA reduced contextual fear conditioning and a post-conditioning injection of NE-enhanced conditioning. These findings are consistent with the idea that amygdala-dependent processes modulate the storage of contextual fear memory. Although our cannula
We suggest that the neurons in the amygdala modulate the storage of the representation of the context in which shock occurred. Three facts support this view. First, there are many reports that rats acquire a representation of an explored context (e.g., Fanselow 1990; Kiernan and Westbrook 1993; Rudy and O'Reilly 1999; Rudy and Wright-Hardesty 2005). Second, inactivating the amygdala neurons with muscimol impairs the consolidation of the memory for an explored context (Huff and Rudy 2004). Third, pre-exposure obscures the modulation effects of the amygdala on contextual fear conditioning.

We, and others have argued that the intact hippocampus supports contextual fear conditioning (as measured by freezing), in part by storing a conjunctive or configural representation of the context (Maren et al. 1997; Fanselow 1999, 2000; Rudy et al. 2004). Several lines of evidence are consistent with this hypothesis (see Barrientos et al. 2002; Rudy et al. 2002; Matus-Amat et al. 2004). If this view is correct, then one could argue that the amygdala is modulating memory processes dependent on the hippocampus. This conclusion is consistent with the literature indicating that the amygdala modulates memory storage processes in the hippocampus (e.g., Packard et al. 1994; Roozendaal and McGaugh 1997b; Roozendaal et al. 1999; Cahill and Packard 2001; McIntyre et al. 2005).

One aspect of our data is inconsistent with the literature. We found that pre-exposing rats to the conditioning context obscured the facilitation of contextual fear conditioning associated with post-conditioning intra-BLA norepinephrine. LaLumiere et al. (2003) found that norepinephrine injected into the amygdala enhanced contextual fear conditioning, even though their rats had been pre-exposed to the conditioning apparatus. It is difficult to know how to account for the differences between our findings and the LaLumiere et al. (2003) data. Their context was markedly different from ours and this may be important. It is also possible that rats pre-exposed to the conditioning context were at a ceiling, so that it would have been difficult to observe any additional influence of norepinephrine on the contextual-fear memory. The ceiling-effect interpretation, if correct, would weaken the theoretical conclusion, based in part on these data, that the amygdala modulates storage of the context memory.

In conclusion, the present data support the hypothesis that the amygdala modulates the storage of a contextual fear memory. Together with the findings of Huff and Rudy (2004) they also support the hypothesis that the amygdala modulates the memory for contextual fear conditioning by influencing the storage of the hippocampus-dependent representation of the conditioning context.

The subjects, adult male Sprague-Dawley rats weighing 250–275 grams at the time of surgery, were housed four to a cage at 25°C on a 12-h light/12-h dark cycle (lights on at 7 a.m.). Rats were allowed free access to food and water. Pre-exposure, contextual-fear conditioning, and testing occurred between 8 and 11:30 a.m. in all experiments. Experiments were conducted in accordance with protocols approved by the University of Colorado Animal Care and Use Committee.

Under halothane anesthesia, rats were placed in stereotactic apparatus (Kopf Instruments) and implanted bilaterally with chronic stainless-steel guide cannulas (Plastics One). The following coordinates, from Paxinos and Watson (1998), were used for bilateral guide cannula implantation, aimed at the BLA region: from bregma, AP −3.0 mm, ML ±4.8 mm, DV −7.5 mm, with internal injection cannula −8.5 mm. Guide cannulas were secured with dental acrylic and three small screws and, to maintain patency, fitted with obturators that extended 1 mm beyond the tip of the guide cannula. Rats were allowed to recover for 7 d.

In Figure 3, (Top) A schematic drawing illustrating the injector-tip placements of subjects injected with vehicle and subjects injected with norepinephrine in Experiment 3. Rats were included in the analysis if the injection sites were in the lateral or basal nuclei of the amygdala. The number of sites appears lower than the number of animals per group because of overlapping sites. This figure was reproduced with permission from Elsevier © 1998, Paxinos and Watson (1998) compact disk edition. From Bregma, the plates span from −1.60 mm to −3.60 mm. (Bottom) Mean percent freezing during Experiment 3 as a function of context pre-exposure and drug. Bars, standard errors of the mean.
A description of the conditioning chamber (Context A) is provided in the Huff and Rudy (2004) study. Context B was a rectangular, hard plastic, opaque rodent cage. The top of the cage was covered in ventilation paper, but the sides were clear hard plastic.

On day 1, rats were transported two at a time from the home cage to Context A (the conditioning chamber) or Context B (rodent cage) in a black bucket with a tight lid. They were then placed in the context and allowed to freely explore for 8 min. The same two rats were then transported back to their home cage in the black bucket.

Twenty-four hours later, rats were taken two at a time in the black bucket to the conditioning chambers. In Experiment 1, rats were in the conditioning context for 30 sec, at the end of which time, a 2-sec, 1.8-mA shock was presented. After the shock, rats were immediately removed and returned to their home cage via the black bucket. In Experiment 2, rats were in the chamber for 60 sec total. After 45 sec, two 0.5-mA shocks were delivered. The shocks were separated by 15 sec. The conditioning parameters varied between experiments to prevent ceiling and floor effects of conditioning from masking the pharmacological treatments. Contextual fear was assessed (as described by Huff and Rudy 2004) 24 h after conditioning.

Microinjections were carried out after contextual-fear conditioning in all experiments. Rats were gently wrapped in a soft towel, and a 33-gauge microinjector (Plastics One) attached to PE50 tubing was inserted through the indwelling guide cannula. The distal end of the PE50 tubing was attached to a 100-µL Hamilton syringe that was attached to a Kopf microinjection unit (Model 5000) that accurately dispensed the desired volume. Bilateral intra-amygdala infusions were in a volume of 0.5 µL per side. In Experiment 1, rats were infused with muscimol or vehicle 15 min post-conditioning. In Experiments 2 and 3, norepinephrine was administered immediately following conditioning.

Muscimol (Sigma Chemical) was dissolved in Ringers Irrigation (United States Pharmacopeia [USP]) for a final concentration of 1 µg/µL. Muscimol or vehicle (ringers solution) was injected bilaterally (0.5 µL/side) into the BLA region over 2 min. Norepinephrine (Sigma Chemical) was dissolved in phosphate buffer for a final concentration of 0.3 µg/0.5 µL, 1.0 µg/0.5 µL, or 3.0 µg/0.5 µL. Phosphate buffer (PBS) or norepinephrine (NE) was injected bilaterally (0.5 µL/side) into the BLA region over 2 min. Rats in all experiments were sacrificed following testing, and their brains were removed and frozen at −20°C until sectioning. The rat brain in stereotaxic coordinates (United States Pharmacopeia [USP]) for a final concentration of 102: 164. 1999. Amygdala modulation of hippocampal-dependent and caudate nucleus-dependent memory processes. Proc. Natl. Acad. Sci. 96: 11642–11647.

References

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