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Amygdala-Mediated Enhancement of Memory for Specific Events Depends on the Hippocampus

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Abstract

Emotional events are often remembered better than neutral events, a type of memory prioritization by affective salience that depends on the amygdala. Studies with rats have indicated that direct activation of the basolateral complex of the amygdala (BLA) can enhance memory for neutral events, and if the activation is brief and temporally targeted, can do so in way that benefits memories for specific events. The essential targets of BLA activation in the case of event-specific memory enhancement were unknown, but the hippocampus was known to receive direct projections from the BLA and to support memory for events. In the present study, rats received counterbalanced infusions of either muscimol, a GABA_A receptor agonist, or saline into the hippocampus prior to performing a novel object recognition memory task during which initial encounters with some of the objects were immediately followed by brief electrical stimulation to the BLA. When memory was tested 1 day later in the saline condition, rats remembered these objects well but showed no memory for objects for which the initial encounter had not been followed by BLA stimulation. In contrast, no benefit to memory of BLA stimulation was observed in the muscimol condition. The results indicated that brief activation of the BLA can prioritize memories for events by enhancing memory for some object encounters but not others and that this benefit to memory depends on interactions between the amygdala and the hippocampus.

Keywords
basolateral amygdala; hippocampus; object recognition memory; memory enhancement; electrical stimulation; fluorophore-conjugated muscimol

1. Introduction

Research with humans and experimental animals has shown that moderate emotional arousal tends to improve memory and that this improvement depends on the basolateral complex of the amygdala (BLA; Cahill & McGaugh, 1998; LaBar & Cabeza, 2006; McGaugh, 2004; Paré, 2003). One important implication is that memories for emotional events are often...
remembered better than neutral events, thereby prioritizing memory by affective salience (Cahill & McGaugh, 1995; McGaugh, 2013). Numerous studies in rats have demonstrated that post-training activation of the BLA enhances memory consolidation when the BLA is activated shortly after the end of the session (Pare, 2003), including memory not typically considered to contain emotional content, such as memory for novel objects (Roozendaal, Castello, Vedana, Barsegyan, & McGaugh, 2008). However, an important unanswered question had been whether brief activation of the BLA could enhance memory for only some events within a learning session, leaving others unaffected so as to maintain the prioritization of the targeted memories.

To address this question, a recent novel object recognition memory study in rats used brief (1 s) electrical stimulation of the BLA immediately following the offset of object exploration for some objects and found that those objects were remembered well one day later (Bass, Partain, & Manns, 2012). In contrast, control objects encountered in the same session were forgotten by that time. The results indicated that activation of the BLA could indeed prioritize memories for specific events, but the essential targets of the BLA activation were unclear as the BLA has broad connections throughout the brain (Krettek & Price, 1977). One primary candidate was the hippocampus, a structure known to receive direct projections from the BLA (Petrovich, Canteras, & Swanson, 2001; Pitkanen, Pikkarainen, Nurminen, & Ylinen, 2000), to show evidence of upregulated synaptic plasticity following BLA activation (Akirav & Richter-levin, 1999; Frey, Bergado-Rosado, Seidenbecher, Pape, & Frey, 2001; Ikegaya, Saito, & Abe, 1995; McIntyre et al., 2005), and to be important for declarative memory (Squire, Stark, & Clark, 2004).

The goal of the present study was to ask whether this BLA-mediated enhancement of memories for specific object encounters depended on the hippocampus. Similar to the previous study (Bass et al., 2012), rats performed a novel object recognition memory task during which brief electrical stimulation of the BLA followed offset of object exploration during the study phase. In addition, during counterbalanced sessions, rats received bilateral infusions of either fluorophore-conjugated muscimol (FCM) or vehicle solution (phosphate-buffered saline, PBS) into the hippocampus prior to the study phase. Muscimol is a GABAA receptor agonist that inhibits pyramidal neuron activity, and by conjugating the drug with fluorophore, the extent of drug diffusion can be estimated under fluorescent microscopy (Allen et al., 2008; Jacobs, Allen, Nguyen, & Fortin, 2013). The results of the present study replicated the previous findings (Bass et al., 2012), and extended the prior work by showing that the BLA-mediated memory enhancement depended on the hippocampus.

2. Material and methods

2.1. Subjects

Seventeen adult male Long-Evans rats were individually housed with free access to water (12-hr light-dark cycle; testing during light phase) and placed on a restricted diet such that they maintained at least 90% of their free-feeding weight. All procedures were approved by the Institutional Animal Care and Use Committee at Emory University.

2.2. Surgery

All rats underwent stereotaxic surgery under isoflurane anesthesia to implant twisted, bipolar stimulating electrodes (platinum, 0.075 mm diameter, teflon insulation; Plastics One, Roanoke, VA) bilaterally in the BLA (3.5 mm posterior, 5.2 mm lateral, and 8.9 mm ventral to bregma) and to implant stainless steel guide cannulae (26 gauge; Plastics One, Roanoke, VA) bilaterally just dorsal to the intermediate hippocampus (5.6 mm posterior, 5.2 mm lateral, and 2.0 mm ventral to bregma). Implants were affixed to the skull with dental
acrylic, and styles were then placed into each cannula and projected 1.5-2.0 mm ventrally past the tip in order to maintain patency of the tube. Rats were allowed to recover for at least 1 week prior to resuming training.

2.3. Electrical stimulation and muscimol infusions

The parameters for electrical stimulation were the same as those previously used by Bass et al. (2012). A hand-held device was used to trigger a 20 μA current from a current generator (S88X Dual Output Square Pulse Stimulator; Grass Technologies, West Warwick, RI) that was delivered unilaterally to the BLA (half left, half right) through a stimulus isolator (SIU-BI Stimulus Isolation Unit; Grass Technologies). The 1-sec stimulation consisted of 8 trains, each with 4 pulses (biphasic square wave pulse width = 500 μs; pulse frequency = 50 Hz; train frequency = 8 Hz). None of the rats showed signs of acute stress (vocalizations, defecation, or freezing) or seizures in response to electrical stimulation.

All infusions were performed under light anesthesia (0.5-2% isoflurane). A volume of 1 μL was infused bilaterally and simultaneously at a rate of 0.25 μL/min (UltraMicroPump; World Precision Instruments, Sarasota FL) through a 33 gauge injection needle that projected 2.0 mm ventral to the tip of the guide cannula. Injection needles remained in place for an additional 2 min following the infusion. Rats were given 30 min to recover from anesthesia prior to testing. Each rat was tested in a counterbalanced manner, once following an infusion of FCM (0.5 μg/μL) and once following an infusion of vehicle (PBS). No infusions were conducted prior to the 1-Day Test Phase.

2.4. Object recognition memory testing

Figure 1 shows the procedure for object recognition memory testing, which was similar to the procedure reported in Bass et al. (2012), with the exception that FCM or PBS was infused bilaterally into the hippocampus prior to the Study Phase in the present study. Rats were trained for 2-3 weeks prior to surgery and for 2 weeks following recovery to complete clockwise laps around a circular track (diameter = 91.5 cm, width = 7 cm) for a small food reward. Objects made of plastic, ceramic, metal, or wood were placed on small platforms on the outside of the track at three possible positions. Rats were permitted to explore objects voluntarily, and a reward was given at the end of the lap irrespective of exploration. Immediately following exploration (within 1 sec) of some objects during the Study Phase, rats received brief electrical stimulation to the BLA (Stimulation objects). Stimulation followed exploration in order to minimize any impact of stimulation on exploration behavior and to align the present experiment with previous studies that targeted memory consolidation. Encounters with Stimulation objects were interleaved with encounters of objects that were not followed by stimulation (No Stimulation objects). Duplicates of both Stimulation objects and No Stimulation objects were then presented along with novel objects (New objects) during the two test phases during which no stimulation was delivered. Memory was tested for half of the objects during the Immediate Test, which occurred immediately following the Study Phase (approximately 45 min after the start of the Study Phase). Memory for the other half of the objects was tested 1 day later on the 1-Day Test.

2.5. Histology

In order to provide an estimate of the extent of diffusion, FCM was again infused into hippocampus several days following the final test session using the same procedure as before. In addition, small electrolytic marking lesions were made at the tips of the BLA stimulating electrodes. Rats were then euthanized with an i.p. injection of euthanasia solution (Euthasol) 75 min after the infusion. Rats were perfused transcardially with saline and formalin, and their brains were extracted and subsequently sliced into 3 interleaved series for staining: cresyl violet for general anatomical landmarks, acetylcholinesterase for
highlighting the basal nucleus in the amygdala, and no stain for visualization of FCM diffusion using a 529 nm – 576 nm light source (peak FCM absorption, 543 nm; Eclipse TE300 Inverted Microscope; Nikon, Melville, NY).

2.6. Video scoring and behavioral data analysis

Using frame-by-frame inspection of video, a rat was judged to be exploring an object if its nose was within 2 cm of the object and the rat was showing evidence of whisking and/or sniffing. Trials on which a rat did not explore the Stimulation object during the Study Phase were excluded as BLA stimulation was to be triggered only at the onset of exploration. This procedure had the potential to artificially increase the exploration times of Stimulation objects relative to other object types. Therefore, only trials in which a rat explored all three object types during the Study Phase were included. Object recognition memory performance during the test phases was calculated as a standard discrimination index that quantified the tendency of rats to explore repeated objects for less time than new objects. Specifically, the mean exploration time for new objects was divided by the sum of the mean exploration time for new and repeated objects [for Stimulation objects: New/(New + Stimulation); for No Stimulation objects: New/(New + No Stimulation)].

3. Results

3.1. Histology and subject exclusion

Figure 2 shows an example of a stimulating electrode marking lesion in the BLA and an example of an FCM infusion in the hippocampus. Five rats were excluded from analyses after histological inspection revealed that either the tip of a stimulating electrode was outside the BLA or the infusion site extended outside the hippocampus. One rat was euthanized prior to completing the experiment due to an infection near the implant site. Testing was discontinued for two more rats after those rats failed to complete all test sessions after several attempts. All rats were excluded or included before analyzing their behavioral data. For the 9 rats included in the final analysis, the stimulating electrodes were all in the BLA in both hemispheres and were positioned near the basal nucleus specifically. For those nine rats, the FCM diffusion appeared to be approximately 1-2 mm in diameter and contained in the hippocampus in both hemispheres, centered near CA2 in the transverse axis (extending to adjacent portions of CA3, CA1, and dentate gyrus) and at an intermediate region of the septal-temporal axis.

3.2. Object recognition memory performance

Figure 3 shows performance on the recognition memory task calculated as a discrimination index. A 2x2x2 (stimulation condition by infusion condition by test phase) repeated measures ANOVA revealed a statistically significant 3-way interaction (F(1,8) = 5.83, p<0.05), a stimulation condition by infusion condition interaction (F(1,8) = 22.07, p<0.01), and a partial effect of infusion (F(1,8) = 7.25, p < 0.05). Closer analysis of the data suggested that in the PBS condition, Stimulation and No Stimulation objects were remembered similarly on the Immediate Test but that only the Stimulation objects were remembered on the 1-Day Test. Specifically, on the Immediate Test, the discrimination index scores for Stimulation and No Stimulation objects following infusions of PBS were similar and both significantly above baseline chance levels (mean ± SEM: Stimulation = 0.57 ± 0.03, t(8) = 2.20, p < 0.05; No Stimulation = 0.57 ± 0.02, t(8) = 3.44, p < 0.01). On the 1-Day Test in the PBS condition, discrimination index scores for only the Stimulation objects were significantly above baseline (mean ± SEM: Stimulation = 0.61 ± 0.03, t(8) = 3.65, p < 0.01; No Stimulation = 0.53 ± 0.03, t(8) = 0.95, p > 0.1), and the discrimination index scores for Stimulation objects were significantly greater than those for No Stimulation objects (t(8) = 2.75, p < 0.05). In comparison to the data from the PBS condition,
discrimination index scores for Stimulation and No Stimulation objects following infusions of FCM were near chance levels on the Immediate Test (mean ± SEM: Stimulation = 0.47 ± 0.03, t(8) = 1.03, p > 0.1; No Stimulation = 0.46 ± 0.04, t(8) = 0.81 p > 0.1). On the 1-Day Test in the FCM condition, the No Stimulation performance was numerically above chance (mean ± SEM = 0.56 ± 0.04), although this difference did not approach statistical significance (t(8) = 1.46 p > 0.1). It is unclear why the performance for this condition was not closer to chance levels, as was the case for both FCM conditions on the Immediate Test. Nevertheless, performance for Stimulation objects on the 1-Day Test in the FCM condition was very clearly at chance levels (mean ± SEM = 0.50 ± 0.03, t(8) = 0.00, p > 0.1). Further, on the 1-Day Test, the discrimination index scores for Stimulation objects under PBS were significantly greater than the scores for Stimulation objects under FCM (t(8) = 2.55, p < 0.05). Thus, the data from the FCM condition indicated that any benefit to memory of BLA stimulation was blocked by inactivation of the hippocampus.

One potential concern was that, during the Study Phase, rats unexpectedly explored Stimulation objects slightly yet significantly more than No Stimulation objects during the PBS (mean ± SEM: Stimulation = 2.43 sec ± 0.21; No Stimulation = 2.09 sec ± 0.24; t(8) = 2.99, p < 0.05) and FCM conditions (mean ± SEM: Stimulation = 2.89 sec ± 0.34; No Stimulation = 2.11 sec ± 0.27; t(8) = 3.67, p < 0.01) and that this extra time might have influenced subsequent memory performance. The cause of these differences was not entirely clear, and the similar memory performance between Stimulation and No Stimulation objects on the Immediate Test would suggest that these slight exploration time differences during the Study Phase were not sufficient to account for the large differences observed between Stimulation and No Stimulation objects on the 1-Day Test in the PBS condition. Nevertheless, to ask whether increased object exploration times during the Study Phase could relate to better memory performance (i.e., decreased exploration times) during the Immediate Test or 1-Day Test, exploration times from the Study Phase were correlated with exploration times from each test for each rat. The resulting Pearson's r values were converted to z scores to permit averaging across rats and to permit t-tests against chance (Z score of 0). No significant negative correlations were observed that would have suggested that the improved test performance generally related to slight increases in Study Phase exploration times. Indeed, the only correlations that were statistically significant were positive correlations, which would strongly suggest that any differences in exploration times during the Study Phase could not account for improved memory for the Stimulation/PBS objects on the 1-Day test (mean Z ± SEM: Stimulation/PBS/Immediate = -0.06 ± 0.29, t(8) = 0.65, p > 0.1; No Stimulation/PBS/Immediate = 0.32 ± 0.35, t(8) = 2.71, p < 0.05; Stimulation/FCM/Immediate = 0.29 ± 0.46, t(8) = 1.85, p = 0.1; No Stimulation/FCM/Immediate = 0.55 ± 0.90, t(8) = 1.85, p = 0.1; Stimulation/PBS/1-Day = 0.24 ± 0.33; t(8) = 2.11, p < 0.1; No Stimulation/PBS/1-Day = 0.31 ± 0.54; t(8) = 1.71, p > 0.1; Stimulation/FCM/1-Day = 0.49 ± 0.32; t(8) = 4.56, p < 0.01; No Stimulation/FCM/1-Day = 0.15 ± 0.35; t(8) = 1.26, p > 0.1).

4. Discussion

The present results replicated and extended prior results from a related study (Bass et al., 2012). In the PBS condition of the present study, brief electrical stimulation to the BLA following offset of exploration of novel objects resulted in those objects being remembered well one day later. In contrast, object encounters not followed by BLA stimulation appeared to be forgotten by that time. Consistent with the idea that BLA activation enhances memory consolidation specifically, BLA stimulation did not appear to influence performance when memory was tested immediately. The results extended the previous results by indicating that the BLA-mediated memory enhancement depended on the hippocampus. Taken together, the results indicated that brief activation of the BLA can prioritize memories for events by
enhancing memory for some object encounters but not others and that this benefit to memory depends on interactions between the amygdala and the hippocampus.

There are direct and indirect connections between the BLA and the hippocampus (Petrovich et al., 2001; Pitkanen et al., 2000), and BLA stimulation can modulate plasticity in cortical areas outside the hippocampus (Chavez, McGaugh, & Weinberger, 2009; Paz, Pelletier, Bauer, & Pare, 2006) as well as in the hippocampus (Akirav & Richter-Levin, 1999; Frey et al., 2001; Ikegaya et al., 1995; Kim, Kim, Park, Cho, & Kim, 2012; McIntyre et al., 2005). A common view is that BLA activation affects signaling cascades related to late-phase LTP in numerous brain regions in support of memory consolidation (Bergado, Lucas, & Richter-Levin, 2011; McGaugh, 2004; Pare, 2003). For example, one previous study found that BLA stimulation following LTP induction in the hippocampus in anesthetized rats had minimal impact on LTP at 1 hour but reinforced maintenance of LTP beyond 1 hour (Frey et al., 2001). In two studies of rats performing an inhibitory avoidance task (Holloway-Erickson, McReynolds, & McIntyre, 2012; McIntyre et al., 2005), activation of noradrenergic receptors in the BLA led to improved memory performance and increased expression of proteins associated with synaptic plasticity (CaMKIIα and/or Arc) in the hippocampus and rostral anterior cingulate cortex. Thus, although the current data suggested that BLA stimulation in the PBS control condition directly or indirectly led to essential changes in hippocampal plasticity-related signaling pathways, the hippocampus may represent one of several potential targets of BLA activation for object recognition memory. In any case, as muscimol is biologically active for only a few hours (Arikan et al., 2002), the impact of BLA stimulation on the hippocampus must have occurred within a few hours of encountering the objects. Indeed, given that Stimulation and No Stimulation objects were often encountered less than a minute apart in the Study Phase, it is likely that these signaling cascades in the hippocampus were triggered by BLA stimulation almost immediately and were rather specific to the particular object encounter.

The similar performance for Stimulation and No Stimulation objects during the Immediate Test in the PBS condition indicates that, although the signaling cascades for upregulated plasticity in the hippocampus were likely triggered rapidly, the benefit to memory was not visible until much later, presumably when the extended process of cellular consolidation (Alberini, 2009; Dudai, 2004) had more fully unfolded. This similar performance also suggests that the BLA stimulation did not lead to any fear-related associations with the objects, as one would have expected rats to avoid these objects on the Immediate Test if that were the case. Indeed, electrical stimulation of the BLA has been shown to be ineffective for inducing conditioned fear in rats (Kim et al., 2013). A second finding from the Immediate Test is that rats did not demonstrate memory for any objects following infusions of FCM. Thus, the role of the hippocampus in the present study was not specific to memory consolidation. Indeed, the present results suggest that, although the hippocampus-dependent enhancement of memory consolidation by activation of the BLA emerges up to a day later, the hippocampus may also play a more general role in memory for object encounters at earlier time points. Future research will be needed to understand how BLA activation can intervene in these hippocampal processes to tag some memories for priority consolidation.

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References


## Highlights

“Amygdala-Mediated Enhancement of Memory for Specific Events Depends on the Hippocampus” By Bass, Nizam, Partain, Wang, and Manns

1. Brief stimulation of basolateral complex of amygdala enhanced recognition memory
2. The memory enhancement was specific to particular object encounters
3. The memory enhancement was apparent one day later but not immediately
4. The memory enhancement depended on the hippocampus
Figure 1.
Schematic of infusion and object recognition memory procedures. Rats were infused with fluorophore-conjugated muscimol (FCM) or control vehicle (phosphate-buffered saline; PBS) 30 minutes prior to testing. In each trial of the Study Phase, rats encountered 3 novel objects such that there was one object from each group of objects (Stimulation objects denoted by an “S”, No Stimulation objects denoted by an “O”, and New objects denoted by an “N”). All objects were presented on individual laps to better isolate the influence of electrical stimulation to the amygdala. Electrical stimulation (denoted by a star) was delivered immediately following encounters with Stimulation objects. Memory for objects from half of the trials was tested during the Immediate Test, and memory for objects from the other half was tested during the 1-Day Test. Corresponding test trials contained duplicates of Stimulation and No Stimulation objects, but New objects were replaced by additional novel objects. All 3 objects were presented together on one lap. No stimulation was delivered during either test phase.
Figure 2.
Example photomicrographs of histology. A. Stimulating electrode localization in the BLA (dashed outline) as shown on tissue stained for acetylcholinesterase. The tips of all electrodes for all rats included in the analyses were contained in the BLA (lateral nucleus, L; basal nucleus, B; accessory basal nucleus, AB). B. Fluorescent image of an infusion of FCM into the hippocampus. Infusions of FCM for all rats included in the analyses appeared to be contained within the hippocampus. The tips of all injection cannulae were localized near CA2 (between CA1 and CA3 fields) in the transverse axis and at an intermediate region of the septal-temporal axis of the hippocampus.
Figure 3. Performance on recognition memory tests following infusions of FCM or control vehicle (PBS) represented as a discrimination index. Following infusions of PBS into the hippocampus, rats demonstrated memory for both groups of repeated objects during the Immediate Test but showed memory for only Stimulation objects on the 1-Day Test. Following infusions of FCM into the hippocampus, rats did not demonstrate memory for objects in either test phase. The dashed line indicates chance performance. Error bars show SEM. * = p < 0.05, ** = p < 0.01, † = p < 0.1.