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Rapid Communication

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 a 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.

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; Pare, 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 1 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

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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 $GABA_A$ 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. Materials and methods

2.1. Subjects

Seventeen adult male Long-Evans rats were individually housed with free access to water (12-h 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 stylets 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-s 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

Fig. 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 s) 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 529–576 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 offset 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)].