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Signature:

Joshua Krasney	Date

Effects of post-study basolateral amygdala noradrenergic activation on long-term object recognition memory in rats

By

Joshua L. Krasney
Master of Arts

Psychology

Joseph Manns, Ph.D.
Advisor

Jocelyne Bachevalier, Ph.D.
Committee Member

Stephan Hamann, Ph.D.
Committee Member

Accepted:

Lisa L. Tedesco, Ph.D.
Dean of James T. Laney School of Graduate Studies

Date

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Joshua L. Krasney
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Abstract

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By Joshua L. Krasney

The basolateral complex of the amygdala (BLA) modulates memory retention of emotional and neutral information, and emotional memories are longer lasting than neutral memories. Yet it is unclear whether BLA activation is involved in producing more stable memories characterized by slower forgetting rates. Additionally, the BLA projects to the hippocampus but preferentially targets the ventral hippocampus more than the dorsal hippocampus. The ventral hippocampus primarily encodes similar events throughout a context whereas the dorsal hippocampus primarily encodes the location in which events occur. Yet it is unclear whether BLA activation preferentially enhances retention of object-in-context information. In the present study, rats were given an object recognition memory task with 1-, 2-, and 3-day study-test delays and post-study BLA activation to investigate BLA modulation of memory stability. Rats were then given an object-in-context recognition memory task with a 1-day study-test delay and post-study BLA activation to investigate BLA-modulated retention of object-in-context information. The results indicated that BLA activation may not enhance memory stability but may enhance retention of object information rather than the predicted object-in-context information.

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A multitude of previous rodent studies have shown that increased activation of the basolateral complex of the amygdala (BLA) can produce memory enhancement of emotional (e.g., LaLumiere, Buen, & McGaugh, 2003) and neutral (e.g., Bass, Partain, & Manns, 2012; Barsegyan, McGaugh, & Roozendaal, 2014) information. BLA activation during an experience can indirectly influence memory by increasing attention and arousal (Phelps & LeDoux, 2005), prompting the use of post-study BLA activation to isolate the effects of the BLA on memory consolidation from these other encoding effects (e.g., Barsegyan et al., 2014). This approach has shown that BLA enhancement of memory retention using behavioral measures are paralleled by changes in synaptic plasticity in other brain regions (Barsegyan et al., 2019). Such findings have led others to suggest that the BLA enhances memory by modulating memory consolidation processes in other brain regions (McGaugh, 2004).

A key question is whether the BLA produces a generic boost in memory strength during memory consolidation or whether the BLA enhances memory retention in particular ways. A way in which the BLA may enhance memory is through enhancing the stability (i.e., slower forgetting) of memories. It is well established that emotional memories are longer lasting than neutral memories (e.g., Cahill & McGaugh, 1995), but it is unclear whether the BLA modulates the stability of emotional memories. Previous studies have combined post-study BLA activation and low-arousal experiences with neutral objects (i.e., novel object recognition tasks) in attempts to determine BLA influences on memory consolidation, but have restricted study-test delays to immediate or 1-day intervals without extending delays (e.g., Bass et al., 2012). A second way in which the BLA may enhance object memory is through retaining event and context associations. The BLA preferentially projects to the ventral hippocampus compared to the dorsal hippocampus (Petrovich, Canteras, & Swanson, 2001), and the ventral hippocampus has been suggested to

encode similar events throughout a context compared to individual locations of events within a context (Komorowski et al., 2013). The use of post-study BLA activation with an object-in-context recognition memory task may determine whether BLA activation preferentially enhances retention of object-in-context information rather than object-in-position or object information. However, a previous study using post-study BLA activation with an object-in-context recognition memory task used naïve rats that may have increased attention and arousal during the study phase (Barsegyan et al., 2014).

Taking together two of the possible benefits of BLA activation addressed above, the current study aimed to determine whether post-study BLA activation (here, via infusion of norepinephrine; NE) can increase memory stability and preferentially enhance object-in-context recognition memory. To address the question of increased memory stability, rats in Experiment 1 underwent an object recognition task with post-study BLA activation and 1-, 2-, and 3-day study-test delays. We predicted that the post-study BLA activation would enhance memory stability as indicated by strong memory performance across the three study-test delays. To address the question of preferential enhancement of object-in-context information, the same rats in Experiment 2 underwent an object-in-context recognition memory task with post-study BLA activation and a 1-day retention test in a repeat and a new context. We predicted that the post-study BLA activation would enhance 1-day object-in-context recognition memory as indicated by strong memory performance in the repeat context but not the new context. The results of the current study suggest that post-study BLA activation may not be sufficient to increase memory stability but may be sufficient to enhance memory retention of object information rather than the predicted object-in-context information from a previous experience.

Experiment 1

Method

Subjects

An *a priori* power analysis determined the use of 12 rats in the current study (G*Power 3.1; Düsseldorf, Germany). The 12 Long-Evans rats (6 males, 6 females) were individually housed (12-hr light-dark cycle; testing during light phase) with unlimited access to water and restricted food diet in which at least 90% of their free-feeding weight was maintained. All procedures involving rats were approved by the Institutional Animal Care and Use Committee at Emory University.

Surgery

Stereotaxic surgery was performed while rats were deeply anesthetized with isoflurane (1-3% in oxygen). Buprenorphine and meloxicam were both provided as preoperative and postoperative analgesics. Stainless steel guide cannulae (26 gauge; Plastics One; Roanoke, VA) were aimed bilaterally at the BLA (3.5 mm posterior, 5.1 mm lateral, 8.4 mm ventral to bregma; Paxinos & Watson, 2007), affixed to the skull with dental acrylic, and covered with stylets protruding 0.2 mm ventral to the tip of the guide cannula. Rats were provided buprenorphine and meloxicam 24 hr after surgery, meloxicam 48 hr after surgery, and a week to recover.

Behavioral Task

Experiment 1 used an object recognition memory task to assess memory performance. Figure 1 shows a schematic of the testing procedure. Prior to experimental testing, rats were habituated to the testing apparatus (52 x 52 cm) for 5 minutes on three consecutive days, followed by one unscored session to familiarize the rats with objects. Objects (~5 x 13 cm) were made of plastic or ceramic. During the study phase, rats were exposed to four identical novel objects (Figure 1). Following the end of the study phase, saline or norepinephrine was infused

into the BLA within 30 minutes (see Infusions below). Testing conditions included 3-minute object exposure followed by NE infusion (3min + NE; experimental norepinephrine), 10-minute object exposure followed by SAL infusion (10min + SAL; experimental control), and 3-minute object exposure followed by SAL infusion (3min + SAL; baseline control). Objects were repositioned (positions of duplicates were swapped) halfway through the 10-minute studies to encourage continued exploration, but not during the 3-minute studies. Each rat was tested twice at 1-, 2-, and 3-day study-test delays for both experimental conditions, and twice at a 1-day study-test delay for the baseline control condition. Rats were tested at only a 1-day study-test delay for the baseline control condition (3min +SAL) because, based on pilot testing, rats were expected to show poor performance even at this shortest delay. During the retention tests, rats were exposed to a pair of repeat objects and a pair of novel objects in a repeat context for 10 minutes (Figure 1). New objects were used for each condition and delay, and the objects and conditions were counterbalanced across rats. Slight alterations to the testing box (e.g., solid black walls vs. black and gray stripes on the walls) were also made between administration of separate conditions to help make each memory condition more distinct. Study or test phase trials in which an object fell were excluded and made up with a new context and object pair. Each test was separated by at least one day. All study and test phase trials were video recorded and scored offline.

Infusions

All post-study infusions were performed under light anesthesia (2.25-2.75% isoflurane) within 30 minutes after training. Norepinephrine (1.0 μg in 0.2 μL; Sigma-Aldrich, St. Louis, MO) was dissolved in saline. In a counterbalanced manner, each rat was bilaterally infused with a volume of 0.2 μL of norepinephrine or saline into the BLA over a period of 30 s

(UltraMicroPump; World Precision Instruments, Sarasota FL), and injection needles remained in place for at least 30 s after infusion (Roozendaal, Castello, Vedana, Barsegyan, & McGaugh., 2008). Injection needles (33 gauge; Plastics One) protruded 1.0 mm ventral to the tip of the guide cannula.

Histology

Several days after the final test, a fluorophore-conjugated molecule (BODIPY-conjugated muscimol; 0.5 μg/μL) was infused to permit post-mortem florescent visualization of the infusion site. Rats were then euthanized via i.p. euthanasia solution (Euthasol), perfused transcardially via PBS and formalin, and their brains were extracted. Brains were sliced and visualized using a fluorescent microscope (peak fluorophore-conjugated muscimol absorption, 543 nm; AxioPlan Upright Fluorescence Microscope; Zeiss, Oberkochen, Germany). Supplementary Figure S1 displays cannula placements for inaccurate (Figure S1A) and accurate (Figure S1B) placement rats.

Video Scoring and Behavioral Data Analysis

Study and test trials were video recorded (Logitech HD Pro C920) and subsequently scored offline. A rat was deemed as exploring an object if his/her nose was with 1 cm of the object and the rat was showing evidence of whisking and/or sniffing. Object recognition performance during the test phase was calculated as a discrimination index (DI) that quantified the amount of time rats explored the repeat objects versus the novel objects. Specifically, the difference of the total exploration of the two novel objects and the total exploration of the two repeat objects was divided by the total exploration time, then multiplied by 100 [([Novel – Repeat]/Total)*100].

Results

After histology, six rats (3 males [Rats 1, 3, 5], 3 females [Rats 8, 10, 12]) were included in the data analysis with bilateral canula placements in the BLA (Figure S1B). The use of only six rats in the data analysis warrants the interpretation of the findings as preliminary.

The rats displayed a similar percent of object exploration time during the study phase in each condition (mean study exploration time (s) ± SEM, % total study time [study exploration time/total study time]; 3min + NE = 21.76 ± 1.60, 12.09%; 10min + SAL = 41.38 ± 3.22, 6.90%; 3min + SAL = 15.24 ± 1.68, 8.47%). Figure 2 shows the DI scores for the 1-, 2-, and 3-day retention tests. Rats in the 10min + SAL condition performed relatively stable throughout the study-test delays (mean DI ± SEM; 1-day = 12.67 ± 12.18; 2-day = 8.46 ± 9.40; 3-day = 10.11 ± 4.73). Conversely, rats in both the 3min + NE and 3min + SAL conditions performed around chance by the 1-day retention test (mean DI ± SEM; 3min + NE = 5.92 ± 6.26; 3min + SAL = -2.36 ± 7.35), and rats in the 3min + NE condition remained around chance performance during the 2-day and 3-day retention tests (mean DI ± SEM; 2-day = 6.69 ± 3.78; 3-day = -2.88 ± 7.40). However, these results were not statistically significant. A 3 x 2 repeated measures ANOVA between the 3min + NE and 10min + SAL experimental conditions showed no main effect of infusion condition ($F(1, 5) = 1.27$, $p = 0.31$, $\eta_p^2 = 0.202$) and no condition by retention test interaction ($F(1, 5) = 0.222$, $p = 0.66$, $\eta_p^2 = 0.043$). Further, rats in the 10min + SAL condition did not perform significantly different from chance at any study-test delay (1-day: $t(5) = 1.274$, $p = 0.26$; 2-day: $t(5) = 0.899$, $p = 0.41$; 3-day: $t(5) = 2.136$, $p = 0.086$) . Supplementary Figure S2 reproduces the data displayed in Figure 2 but includes all 11 rats that were tested (6 accurate cannula placements, 5 missed placements; 1 rat never tested due to illness). A 3 x 2 repeated measures ANOVA between the 3min + NE and 10min + SAL experimental conditions including all 11 rats showed no main effect of infusion condition ($F(1, 10) = 3.05$, $p = 0.11$, $\eta_p^2 = 0.234$)

and no condition by retention test interaction ($F(1, 10) = 0.279$, $p = 0.61$, $\eta_p^2 = 0.027$). Additionally, there was no significant difference between the 3min + NE condition for the inaccurate placement rats (mean DI ± SEM; 1-day = 24.36 ± 9.61; 2-day = 4.29 ± 9.12; 3-day = 14.11 ± 5.10) and the accurate placement rats (mean ± SEM; 1-day = 5.92 ± 6.26; 2-day = 6.69 ± 3.78; 3-day = -2.88 ± 7.40; $F(1,4) = 0.021$, $p = 0.892$; $\eta_p^2 = 0.005$; FigureS3). These data suggest that the shorter duration of exploration followed by increased amygdala activity via norepinephrine (3min + NE) did not improve memory retention for any of the study-test delays compared to more exploration (10min + SAL).

To determine the potential influence of sex on memory retention in our task, an exploratory analysis was conducted in which the data were split between males (n = 3) and females (n = 3). Figure 3 shows the DI scores of for the 1-, 2-, and 3-day retention tests separately for males (Figure 3A) and females (Figure 3B). In the 3min + NE condition, male rats displayed a gradual decline in memory performance across the three retention tests (Figure 3A; mean DI ± SEM; 1-day = 14.24 ± 10.23; 2-day = 11.85 ± 5.83; 3-day = 5.70 ± 4.95), whereas female rats performed at or below chance across the three retention tests (Figure 3B; mean DI ± SEM; 1-day = -2.39 ± 4.67; 2-day = 1.53 ± 3.27; 3-day = -11.47 ± 13.26). The male and female rats showed opposite trends in the 10min + SAL condition: males performed better during the 1-day retention test followed by chance performance during the 2-day and 3-day retention tests, whereas females performed at chance performance during the 1-day retention test followed by better performance on the 2-day and 3-day retention tests (Figure 3A, 3B). A 3 x 2 x 2 repeated measures ANOVA between 3min + NE and 10min + SAL experimental conditions showed no condition by retention test by sex interaction ($F(1, 4) = 4.46$, $p = 0.10$, $\eta_p^2 = 0.527$). Further, only male rats at a 1-day study-test delay in the 10min + SAL condition ($t(2) = 10.9$, $p = 0.009$)

and female rats at a 2-day study-test delay in the 10min + SAL condition ($t(2) = 10.2$, $p = 0.009$) performed significantly different than chance (Figure 3A, 3B).

Interim Summary

The pattern of results from Experiment 1 showed that longer study exploration (10min + SAL) produced a relatively slower rate of forgetting compared to shorter exploration accompanied by a post-study increase in amygdala activity (3min + NE). Thus, contrary to our initial prediction, the results suggest that increased post-study activation of the amygdala via NE did not slow the rate of forgetting for object recognition memory compared to longer study exploration. It is well established that emotional information produces slower forgetting compared to neutral information, and that amygdala activation during memory consolidation (i.e., post-study) is involved with enhancement of memory retention for emotional (LaLumiere et al., 2003) and neutral (Roozendaal et al., 2008) information. Accordingly, we predicted that post-study BLA activation, a time that does not influence encoding, might be a mechanism involved in producing slower rates of forgetting. However, the current results do not provide support for this prediction. Indeed, although underpowered, the mean performance of the 3min + NE condition was lower than the 10min + SAL control condition at each timepoint, especially at the 3-day retention test. The consistently lower mean performance for the 3min + NE condition suggests that even with more subjects, the mean performance of the 3min + NE condition would not increase to be statistically higher than the 10min + SAL condition. Regardless, the initial prediction of amygdala activation producing a slower forgetting rate would likely not be supported even with more subjects. There are many differences between targeted post-study BLA activation used in the current experiment and widespread physiological changes that occur in emotional experiences, differences that will be considered further in the General Discussion.

At the time of completing data collection for all 11 rats tested in Experiment 1, the data had suggested that the 1-day DIs for the 3min + NE and 10min + SAL conditions were similar (i.e., guide cannula placements had not yet determined; see Figure S2 for a plot of the results for all 11 rats). Based on this similar 1-day memory performance between conditions, Experiment 2 was next conducted to ask if 1-day memory might differ between the two conditions in terms of the extent to which the memory was sensitive to a change in the appearance of the testing apparatus. In particular, the question of interest for Experiment 2 was whether post-study BLA activation led memory for the objects to be more or less associated with memory for the spatial context in which the objects were initially encountered.

Experiment 2

Method

Subjects

Five rats (3 males [Rats 1, 3, 5], 2 females [Rats 8, 12]) from Experiment 1 with accurate cannulae placements were included in the data analysis for Experiment 2. Experiment 2 did not investigate sex differences due to the small number of female subjects.

Behavioral Task

Experiment 2 used an object-in-context recognition memory task to determine the influence of increased BLA activation on context-dependent versus context-independent memories. Figure 4 shows a schematic of the testing procedure. During the study phase, rats were exposed to two pairs of novel objects (Figure 4). Similar to Experiment 1, testing conditions included 3 minutes of exploration followed by NE infusion into the BLA (3min + NE) and 10 minutes of exploration followed by SAL infusion into the BLA (10min + SAL). Infusions occurred within 30 minutes of completing the study phase. After a 1-day study-test

delay, rats explored a repeat context and a new context, each containing a pair of repeat objects and a pair of novel objects (Figure 4). Each rat performed each condition once. New objects and contexts were used for each condition and delay, and the objects and conditions were counterbalanced across rats. Contexts were altered by using floors with unique designs and textures, changing the configuration of panels on the walls of the testing apparatus, and changing the location of the testing apparatus within the testing room. Study or test phase trials in which an object fell were excluded and made up with a new context and object pair. Each study-test administration was separated by at least one day. All Study and Test Phase trials were video recorded and scored offline.

Infusions

See Infusions section in Experiment 1 Method.

Histology

See Histology section in Experiment 1 Method.

Video Scoring and Behavioral Data Analysis

Study and test trials were video recorded and subsequently scored offline. Video scoring criteria and DI calculations were similar to Experiment 1. However, in Experiment 2, a DI was calculated for the repeat context and the new context for each condition, with each context containing a pair of repeat objects and a pair of novel objects (Figure 4). Specifically, the DI was calculated in each context as the difference of the total exploration of the two novel objects and the two repeat objects divided by the total exploration time, then multiplied by 100 [(Novel – Repeat)/Total)*100].

Results

Experiment 2 included five rats (3 males, 2 females) in the data analysis, which warrants the interpretation of the findings as preliminary.

Figure 5 shows the DI scores for the 1-day object-in-context recognition memory for both conditions in each context. Rats in both testing conditions performed well in the repeat context (mean DI ± SEM; 3min + NE = 28.26 ± 10.82; 10min + SAL = 20.12 ± 16.31), whereas only rats in the 3min + NE condition performed well in the new context (mean DI ± SEM; 3min + NE = 19.45 ± 15.75; 10min + SAL = -3.89 ± 11.52). However, due to the small sample size, these trends were not statistically significant. A 2 x 2 repeated measures ANOVA revealed no interaction of condition by context ($F(1, 4) = 1.068$, $p = 0.36$, $\eta_p^2 = 0.211$). Further, neither condition performed significantly different from chance in either the repeat (3min + NE: $t(4) = 2.61$, $p = 0.059$; 10min + SAL: $t(4) = 1.23$, $p = 0.29$; Figure 5) or the new context (3min + NE: $t(4) = 1.23$, $p = 0.29$; 10min + SAL: $t(4) = -0.337$, $p = 0.75$; Figure 5). Supplementary Figure S4 reproduces the data shown in Figure 5 but includes all 9 rats that were tested (5 accurate placements, 4 missed placements). Including all of the rats produced a similar trend as Figure 5: both conditions performed well in the repeat context, whereas only the 3min + NE condition performed well in the new context (Figure S4). Accurate placement rats (n = 6) performed better than the inaccurate placement rats (n = 5) during the 3min + NE condition in the repeat context (mean ± SEM; accurate placement rats = 28.26 ± 10.82; inaccurate placement rats = 10.22 ± 14.26), whereas both groups performed similarly in the new context (mean ± SEM; accurate placement rats = 19.45 ± 15.75; inaccurate placement rats = 21.40 ± 23.21). A 2 x 2 repeated measures ANOVA revealed no interaction of placement accuracy by context ($F(1, 3) = 1.34$, $p = 0.33$, $\eta_p^2 = 0.309$; Figure S5). These data provide preliminary evidence that increased amygdala activity via NE enhanced retention of object information from a previous experience, whereas

increased exposure enhanced retention of object-in-context information in the current paradigm. The low power in the current study warrants more subjects to further support the trends of Experiment 2.

Interim Summary

The pattern of results from Experiment 2 showed that both amygdala activation (3min + NE) and longer study exploration (10min + SAL) produced relatively strong 1-day memory performance in the repeat context, whereas only the amygdala activation condition produced strong memory performance in the new context. These results suggest that the primary benefit of amygdala activation was enhancing 1-day memory performance in the new context relative to the longer study exploration condition. Accordingly, the amygdala activation enhanced retention of object information more than object-in-context information. Subsequently, presentation of the repeat object in either the repeat or new context produced strong memory performance. Conversely, the longer study exploration enhanced retention of object-in-context information more than object information. Subsequently, presentation of the repeat object in only the previous context produced strong memory performance. The results of the control group are consistent with a previous study that found enhanced object-in-context recognition memory performance for a 10min + SAL control group during a similar task (Barsegyan et al., 2014).

The BLA projects to many regions of the hippocampal-dependent memory system and modulates memory consolidation of many types of information based on the information processed in the downstream projections (McGaugh, 2004). One region of the hippocampal-dependent memory system that receives input from the BLA is the hippocampus in which the ventral hippocampus is preferentially innervated compared to the dorsal hippocampus (Petrovich et al., 2001). Evidence suggests that pyramidal cells in the ventral hippocampus represent

related events throughout a context, whereas pyramidal cells in the dorsal hippocampus represent specific locations of events within a context (Komorowski et al., 2013). Accordingly, we predicted that post-study BLA activation would enhance object-in-context recognition memory retention through modulating consolidation processes in the ventral hippocampus, leading to strong recognition memory performance restricted to the context of the previous experience (i.e., the repeat context). Contrary to our prediction, the evidence suggests that the BLA activation enhanced recognition memory performance in both the repeat context and the new context, suggesting enhanced memory retention of object information from the previous experience rather than object-in-context information. As stated, the BLA projects to many regions of the hippocampal-dependent memory system, and norepinephrine-induced activation of the BLA is not projection-specific. Hence, we will discuss potential BLA projections that might result in preferential memory enhancement of object information as opposed to object-in-context information.

The BLA projects to the perirhinal cortex (PRC; Collins, Pelletier, & Paré, 2001), and convergent evidence from various techniques and species supports the role of the PRC in recognition memory. Previous studies have shown that PRC lesions produce recognition memory impairments for nonhuman primates (e.g., Meunier, Bachevalier, Mishkin, & Murray, 1993) and rats (e.g., Wiig & Burwell, 1998). More recently, studies have shown that PRC activation via optogenetic stimulation influences recognition memory performance in nonhuman primates (Tamura et al., 2017) and rats (Ho et al., 2015) by modulating the level of object familiarity. Indeed, the optogenetic studies suggest that both object novelty and object familiarity are encoded in the PRC and can be manipulated based on the location and frequency of PRC activation. Another study in rats that used single-unit electrophysiological recordings

showed that PRC neurons encode one or more objects throughout an environment, but encode relatively sparse spatial information (Deshmuhk, Johnson, & Knierim, 2012). In a human fMRI study, PRC activity during encoding was associated with acquisition of item representations that support subsequent item recognition performance, but PRC activity did not reflect acquisition of conjunctive item and context representations that support subsequent source recollection performance (Davachi, Mitchell, & Wagner, 2003). In the current experiment, the post-study BLA activation may have enhanced activity in the PRC, in turn strengthening the consolidation of the object representations from the study phase. Consequently, presenting the repeat objects in either the repeat context or the new context during the test phase elicited retrieval of the consolidated object representations in the BLA activation condition.

The BLA also projects to the lateral entorhinal cortex (LEC; Colino & Fernández de Molina, 1986; Pitkänen, Kelly, & Amaral, 2002), and previous studies have suggested that the LEC encodes both object and spatial information. The LEC receives input from the PRC, and single-unit electrophysiological recordings in rats have shown similar proportions of neurons that respond to objects in the LEC and the PRC (Deshmuhk et al., 2012). However, neurons in the LEC, but not the PRC, have displayed object-in-position coding, and can respond to previous object positions after the objects have been moved (Deshmuhk et al., 2012; Tsao, Moser, & Moser, 2013). Moreover, the LEC has been shown to encode contextual (Tsao et al., 2018) and object-in-context information (Wilson et al., 2013). Another study that recorded from both the PRC and the LEC found that both regions similarly encode object and object-in-position information, and both similarly encode contextual information to a lesser extent (Keene et al., 2016). It is worth noting that the objects in the study by Keene and colleagues (2016) were bowls containing a food reward, which may have increased the salience of and attention towards

the objects. Consequently, the levels of encoding object, object-in-position, and contextual information may be influenced by the relative salience of object and context information, like whether the objects are rewarded or non-rewarded.

Given that the current task used non-rewarded objects, and that LEC activity in previous studies using non-rewarded objects have shown encoding of object-in-context information, we suggest that the BLA activation in the current experiment likely enhanced object recognition memory via increasing PRC activity more than LEC or ventral hippocampal activity. Although the BLA projects elsewhere, the regions previously discussed are likely candidates of the results from post-study manipulation of the BLA.

General Discussion

The results from Experiments 1 and 2 together suggest that post-study amygdala activation via norepinephrine enhanced memory retention of object representations of a previous experience but may or may not produce more stable memories. There are several potential explanations why the amygdala activation condition did not exhibit more stable memories in Experiment 1. It may be the case that the procedure of 3-minute object exploration followed by post-study activation of the amygdala via 5 μg/μL of NE was not the precise combination of exposure time and BLA activation required for amygdala enhancement of memory stability. If so, minor changes to this combination like using a different concentration of NE or using a slightly longer duration of study exposure may be sufficient for the amygdala to enhance memory stability. Alternatively, amygdala activation during the experience (i.e., the study phase) itself may be required to produce more stable memories. As such, the combination of NE concentration and study exposure time used in Experiment 1 may be sufficient to enhance memory stability, but administration of NE during the experience may be necessary. In contrast

to these minor procedural changes, it may be the case that other components of emotional memories aside from or along with amygdala activation are involved in producing stable memories. Emotional experiences produce widespread physiological changes (McGaugh, 2015). Amygdala activation during emotional experiences may require interactions with other components of the experience to produce stable memories, such as increased arousal, increased attention, and/or increased local activity in regions of the hippocampal-dependent memory system. Conversely, some of these other components of emotional experiences may be sufficient to produce more stable memories without requiring amygdala activation. Future research would benefit from determining which aspects of emotional experiences produce more stable memories.

The Experiment 1 results also suggest a potential sex difference in the longer study exploration condition: male rats displayed relatively high memory performance at 1 day followed by chance performance at 2 and 3 days, whereas female rats displayed chance performance at 1 day followed by relatively high performance at 2 and 3 days. We predicted that there would not be sex differences in object recognition memory due to using neutral objects and a relatively low-arousal task. A previous study in humans using neutral information with a post-learning stressor to induce cortisol release showed memory improvement for males but not females, similar to the current study (Andreano, & Cahill, 2006). A follow-up study showed interactions between females' menstrual phase and the impact of a post-learning stressor on memory enhancement (Andreano, Arjomandi, & Cahill, 2008). Future studies are needed to determine the robustness of the trend in sex differences observed in the current study. Moreover, future studies would benefit from recording the estrus cycle of female rats to determine potential interactions between menstrual phase and post-learning amygdala activation for memory enhancement.

The Experiment 2 results suggest that post-study amygdala activation via norepinephrine during the current object-in-context recognition memory task enhanced 1-day memory retention for object representations, potentially by increasing activity in the perirhinal cortex as previously discussed. In contrast, the longer study exposure time enhanced object-in-context memory retention. The information that will undergo memory consolidation must first be encoded during the experience. Two factors that influence the information that is encoded during the experience are its relative salience and the duration of the experience, the latter of which influences the likelihood of encountering the information. As suggested in the Experiment 2 Interim Summary, the relative salience of the object and the context may influence the levels of encoding object, object-in-position, and object-in-context information. For instance, a recent study using a similar paradigm found that both 3min + NE and 10min + SAL conditions, like the current experiment, enhanced 1-day object-in-context recognition memory when using rats that were not habituated to the testing apparatus (Barsegyan et al., 2014). Along with heightened arousal and attention, the naïve rats in this previous study likely found both contextual and object information to be salient, as the rats had not previously experienced the testing apparatus or the objects. In comparison, the rats in the current experiment had encountered many similar contexts in Experiment 1, possibly making the distinctive objects more salient information than the relatively minor contextual changes. Consequently, rats in the 3min + NE condition in the current experiment possibly spent more of their study phase encoding the relatively more salient objects versus the context, leading to stronger memory consolidation of object information. Conversely, rats in the 10min + SAL condition in the current experiment possibly had adequate time to encode the relatively salient objects along with the minor changes in context, leading to the enhanced retention of a conjunctive object-in-context representation. The current experiment

quantified object encoding but not context encoding. Future studies would benefit from operationalizing both object and context exploration, and comparing these exploration times to subsequent object-in-context recognition memory performance. Moreover, future studies would benefit from investigating whether post-study amygdala activation in the current paradigm preferentially enhances memory retention of object representations even during 10-minute study exposure, a study duration that would permit more encoding of contextual information. Finally, future studies would benefit from combining post-study amygdala activation and PRC inactivation to determine whether the PRC influenced the amygdala-mediated object recognition memory enhancement in the current experiment.

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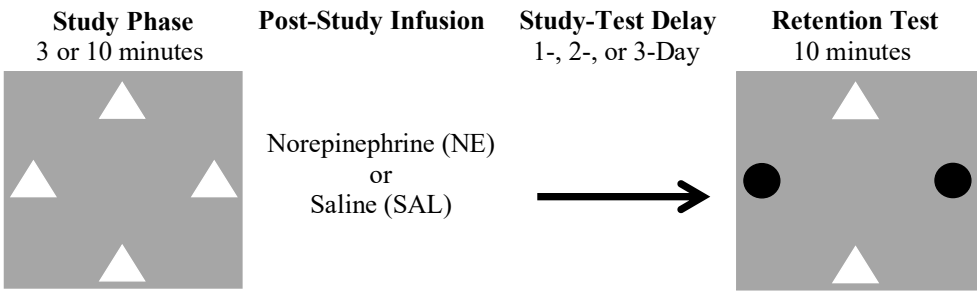


Figure 1. Schematic of the object recognition task. Rats were exposed to four identical objects during the study phase for three or ten minutes followed by an infusion of norepinephrine or saline into the BLA: 3min + NE (experimental norepinephrine), 10min + SAL (experimental control), or 3min + SAL (baseline control). After 1-, 2-, or 3-Day study-test delays, rats were exposed for ten minutes to a pair of repeat objects and a pair of novel objects placed across from each other.

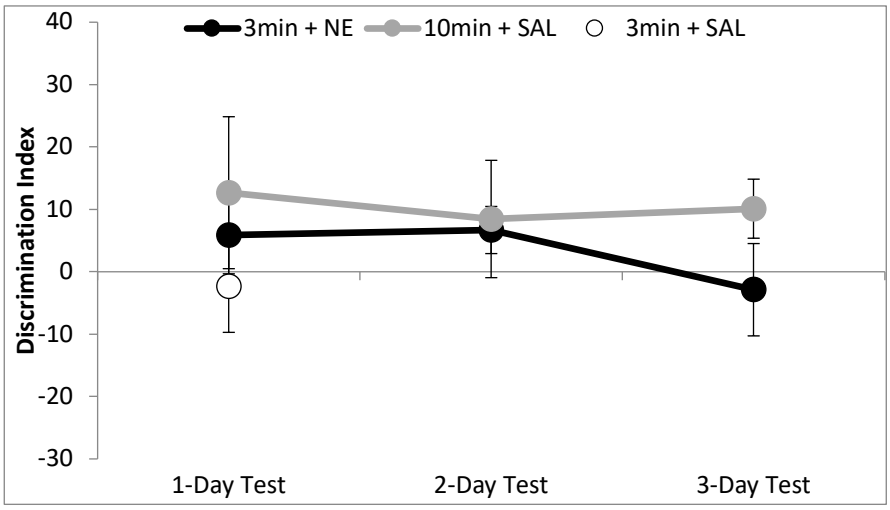


Figure 2. Recognition memory performance displayed as discrimination index (*n* = 6). Rats performed better throughout the study-test delays with more study exploration and no post-study increase of amygdala activity (10min + SAL) versus less study exploration and post-study increase of amygdala activity (3min + NE). Rats in the baseline control condition (3min + SAL) performed at chance performance. Discrimination index of 0 is chance performance. Error bars show SEM.

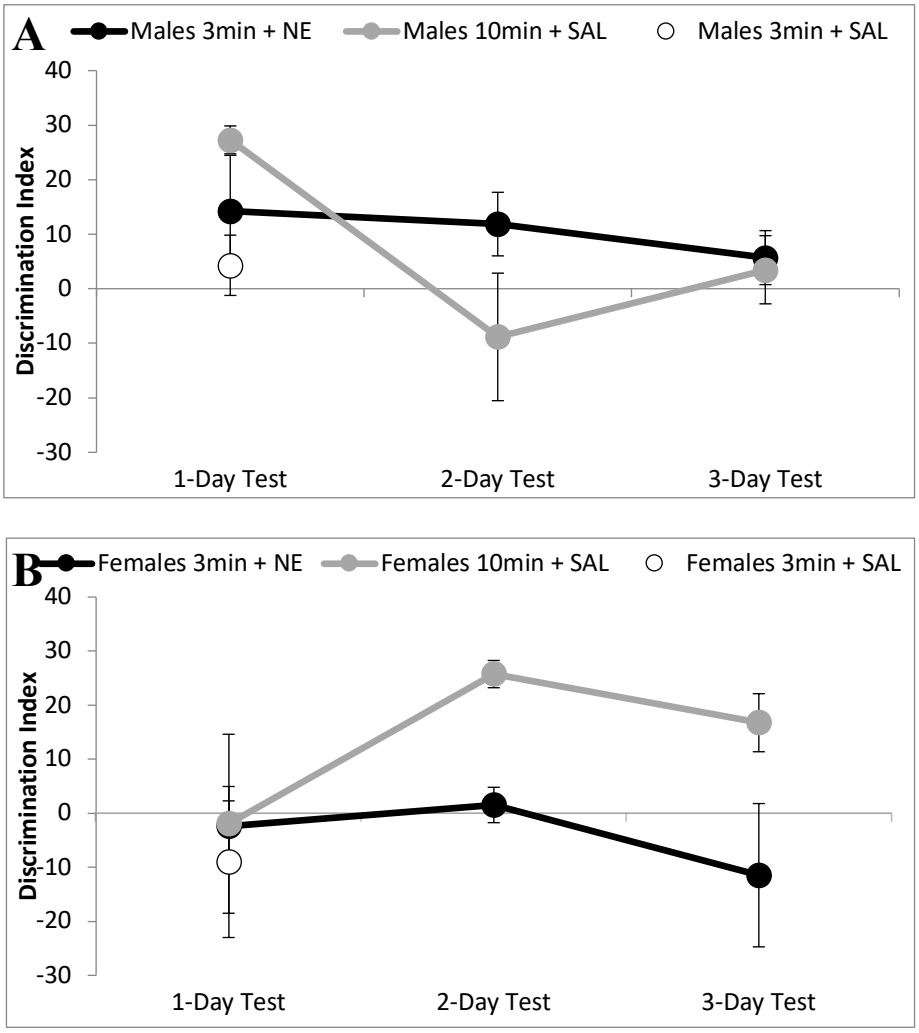


Figure 3. Recognition memory performance displayed as discrimination index for males (n = 3) and females (n = 3). A. Performance for male rats was relatively stable across the three study-test delays in the 3min + NE condition, but decreased to around chance after one day in the 10min + SAL condition. Male rats in the baseline control condition (3min + SAL) performed around chance. B. Performance for female rats at one day was around chance for both 10min + SAL and 3min + NE conditions, but remained around chance in the 3min + NE condition while showing relative increase during the following study-test delays in the 10min + SAL condition. Female rats in the baseline control condition

(3min + SAL) performed below chance. Discrimination index of 0 is chance performance. Error bars show SEM.

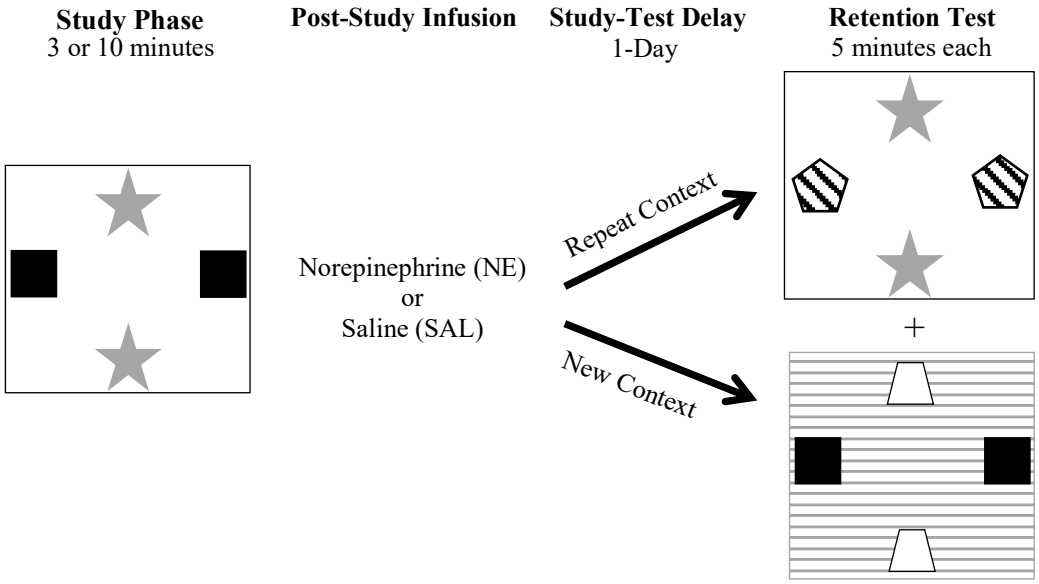


Figure 4. Schematic of the object-in-context recognition task. Rats were exposed to two pairs of identical objects during the study phase for three or ten minutes followed by an infusion of norepinephrine or saline into the BLA: 3min + NE (experimental norepinephrine) or 10min + SAL (experimental control). After a 1-Day study-test delay, rats were exposed to a repeat context and a new context for five minutes each, with both contexts containing a pair of repeat objects in the same location as the study phase and a pair of novel objects replacing the other two object locations.

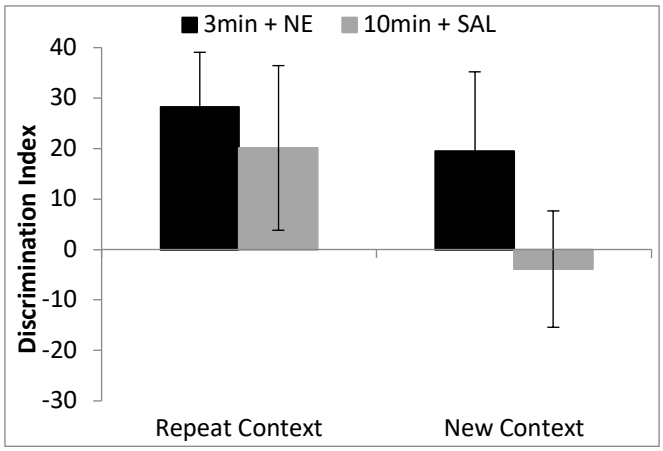
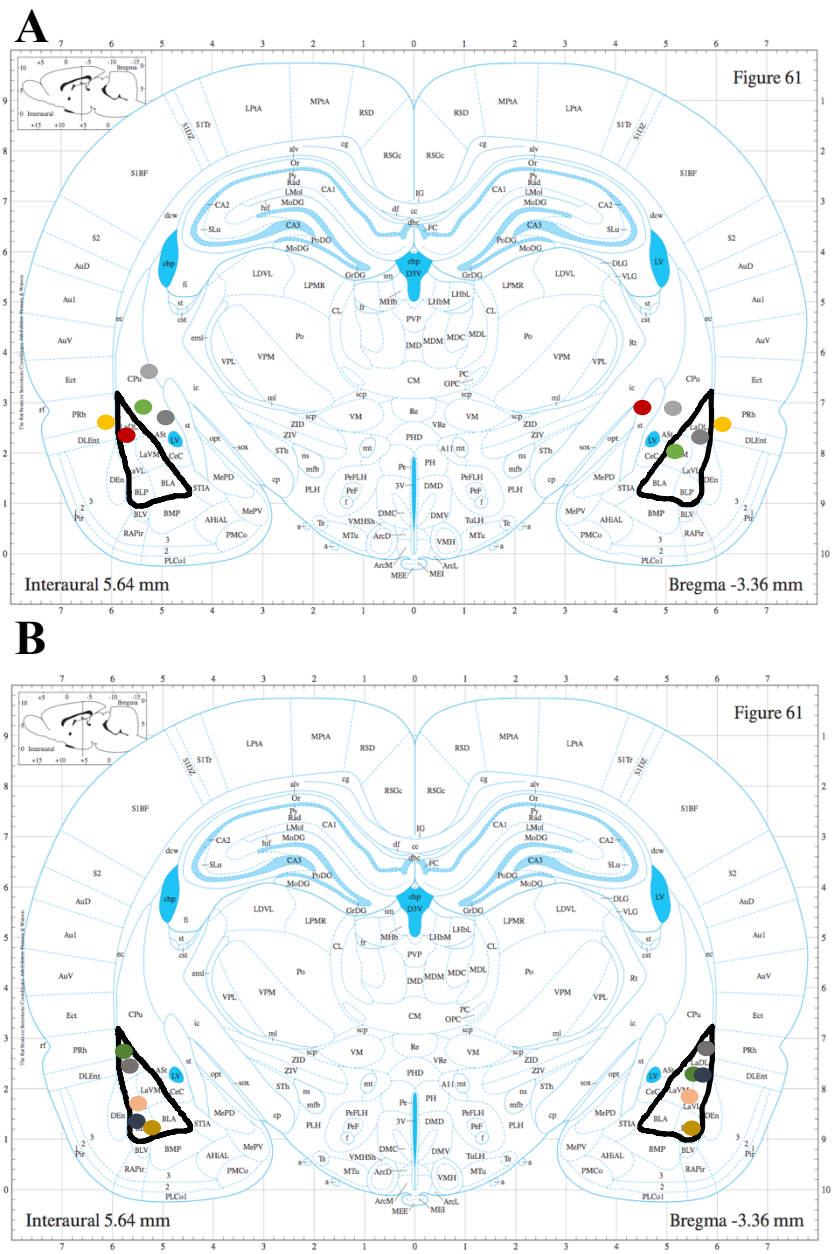
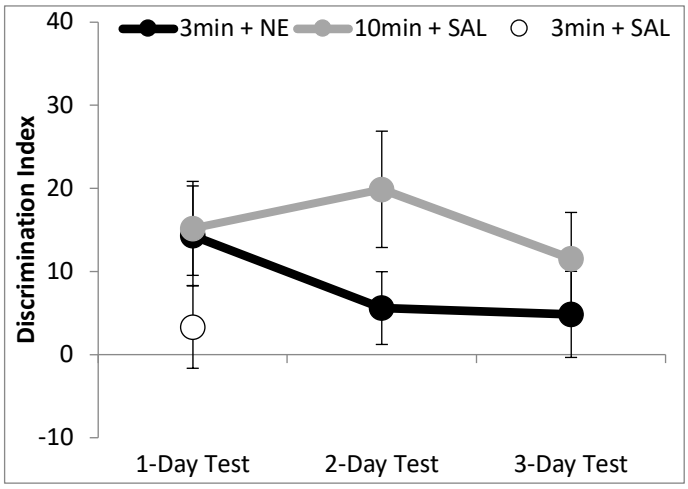


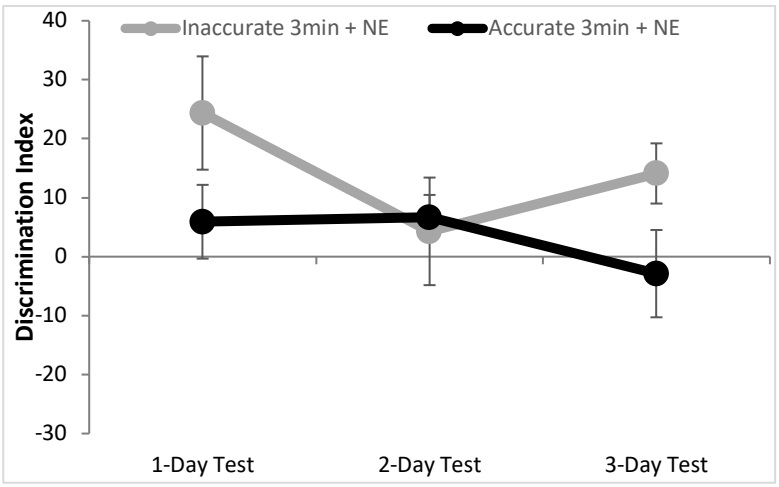
Figure 5. Object-in-context recognition memory performance displayed as discrimination index (n = 5). After the 1-Day study-test delay, rats remembered repeated objects better across the repeat and new contexts in the 3min + NE condition versus the 10min + SAL condition. Rats in the 10min + SAL condition performed well in the repeat context, but around chance in the new context. Discrimination index of 0 is chance performance. Error bars show SEM.



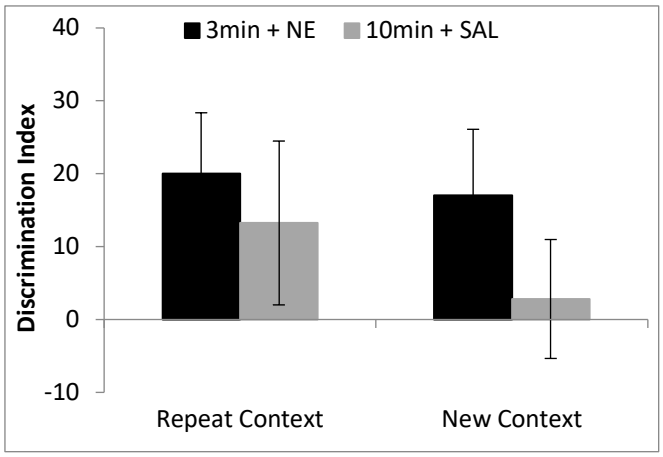
Supplemental Figure 1. Placements of guide cannulae. A. Placements of guide cannulae for inaccurate placement rats (n = 5). Each dot indicates the tip of a guide cannula, and colors represent individual rats. The dots are superimposed on a rat brain atlas (Paxinos & Watson, 2007). The BLA is outlined in black. B. Same as A, but for accurate placement rats (n = 6). Note that accurate placement rats required the tip of the guide cannula within the BLA in both hemispheres.



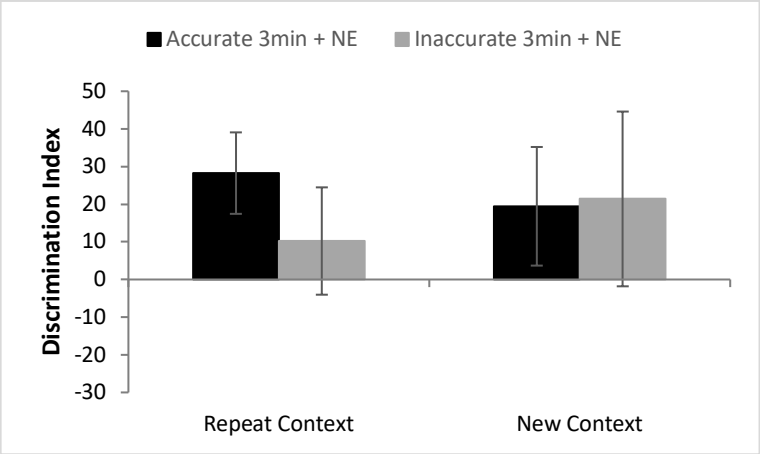
Supplemental Figure 2. Recognition memory performance displayed as discrimination index (n = 11). When including all rats (i.e., inaccurate placements and accurate placements), rats performed similarly well on the 1-Day retention test in both the 3min + NE and 10min + SAL conditions, while rats in the baseline control condition (3min + SAL) performed around chance. Rats in the 10min + SAL condition performed relatively stable throughout study-test delays, while rats in the 3min + NE condition decreased performance to around chance beyond the 1-Day Test. Discrimination index of 0 is chance performance. Error bars show SEM.



Supplemental Figure 3. Recognition memory performance displayed as discrimination index for inaccurate placement rats (n = 5) and accurate placement rats (n = 6) during the 3min + NE condition. The accurate placement rats performed around chance at all three study-test delays. Conversely, the inaccurate placement rats performed relatively well at the 1-day retention test and performed worse during the 2- and 3-day retention tests. The trends were not statistically different between the two groups (See Experiment 1 Results). Discrimination index of 0 is chance performance. Error bars show SEM.



Supplemental Figure 4. Object-in-context recognition memory performance displayed as discrimination index (n = 11). When including all rats (i.e., inaccurate placements and accurate placements), rats performed better in the 3min + NE condition versus 10min + SAL condition across the repeat and new contexts. Rats in the 10min + SAL condition performed well in the repeat context, but around chance in the new context. Discrimination index of 0 is chance performance. Error bars show SEM.



Supplemental Figure 5. Object-in-context recognition memory performance displayed as discrimination index for inaccurate placement rats (n = 5) and accurate placement rats (n = 6) during the 3min + NE condition. The accurate placement rats performed better in the repeat context than the inaccurate placement rats, whereas the two groups performed similarly in the new context. The trends were not statistically different between the two groups (See Experiment 2 Results). Discrimination index of 0 is chance performance. Error bars show SEM.