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The Amygdala Modulates Declarative Memory for a Novel Object Recognition Task

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Abstract

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Emotionally arousing events are remembered better than neutral events, and projections from the amygdala to the hippocampal memory system are thought to be a central pathway in the neural circuitry underlying this enhancement for emotional memory. However, it is not known precisely how the amygdala modulates neural activity in the hippocampal memory system to facilitate ongoing processes related to memory storage. The goal of our project was to better understand how the amygdala modulates hippocampal-dependent, declarative memory. Previous studies have successfully modulated declarative memory through electrical and pharmacological stimulation; however, these paradigms involved prolonged activation of the amygdala for the entire training session. The present study addresses this issue by refining a method of very briefly electrically stimulating the amygdala with precise timing in hopes of targeting the neural connection between the basal nucleus of the basolateral complex of the amygdala and the hippocampus at only the moments in which the to-be-enhanced objects are encountered by the rats. Rats performed a novel object recognition task in which they freely examined non-stimulated objects and objects paired with electrical stimulation. The main finding of the present novel object recognition task is that after a one day retention period, rats remembered objects paired with stimulation; whereas, rats did not remember objects not paired with stimulation after a one day retention period.

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Introduction

The hippocampal memory system supports declarative memory, the ability to remember factual information and events (Squire, 1992). Declarative memory can be modulated by emotion, and emotionally arousing events are often remembered better than neutral events (Bohannon, 1988). Projections from the amygdala to the hippocampal memory system are thought to be a central pathway in the neural circuitry underlying this enhancement for emotional memory (Cahill et al., 1995; Cahill & McGaugh, 1998; McGaugh, 2004), and the basolateral nucleus of the amygdala selectively mediates the memory-modulating effects (Cahill & McGaugh, 1998; McGaugh, 2004). Though much is known regarding the influence of the amygdala over the hippocampal memory system, there is a significant gap in our understanding of the precise neural mechanisms through which the amygdala modulates neural activity in the hippocampal memory system to facilitate memory consolidation. Additionally, few studies have addressed the modulation of declarative memory for neutral events by momentary activation of the basolateral complex of the amygdala. The present study utilizes a refined method of electrically stimulating the amygdala in hopes of targeting the neural connection between the basal nucleus of the basolateral complex of the amygdala and the hippocampus. The expectation is that precisely timed electrical stimulation of the basal nucleus should enhance the declarative memory for objects during a novel object recognition task.

Enhancement of Declarative Memory for Arousing Events

For many years, everyday experience and experimental evidence have supported the idea that emotionally arousing events tend to be recalled more accurately, more readily, and for a longer time than an experience considered to be neutral or less arousing (Cahill, 2000). In 1919, G.M. Stratton stated for an emotionally arousing event that, "The person recalls in almost photographic detail the total situation at the moment of shock, the expression of face, the words uttered, the position, garments, pattern of carpet, recalls them years after as though they were the experience of yesterday" (Stratton, 1919). Although it is now known that the enhanced memory does not come in the form of a perfect record of the emotional event, there is much data to support the view that emotional material is often remembered better than neutral material (Aldolphs et al., 1997; Cahill et al., 1995; Cahill et al., 1996; Cahill & McGaugh, 1998; McGaugh, 2004). Experimental evidence of the amygdala's modulation in declarative memory includes both animal and human studies (Aldolphs et al., 1997; Cahill et al., 1995; Cahill et al., 1996; Cahill & McGaugh, 1998; Gold et al., 1975; McGaugh, 2004). During an inhibitory avoidance task, electrical stimulation of the amygdala in rats enhanced declarative memory when paired with a light footshock and impaired declarative memory when paired with an intense footshock (Gold et al., 1975). Later, human studies found that emotionally arousing stimuli were better remembered than neutral stimuli, and the amygdala supports the enhancement of declarative memory for emotionally arousing events (Cahill et al., 1996; Aldolphs et al., 1997; Hamann et al., 1997).

Amygdala Activity Increases with Acquisition of Declarative Memory for Emotionally Arousing Stimuli

Human brain imaging studies provide extensive evidence supporting that activation of the amygdala correlates with the enhancement of emotional memory (Cahill, 2000). Participants received positron emission tomography scans while viewing neutral and emotionally arousing films (Cahill et al., 1996). Participants were requested to attend to the films and rated the amount of emotion evoked by each film. Participants were not informed that they would later be asked to recall details of the stimuli. Three weeks after viewing the stimuli, participants were given a free recall test. Participants recalled more emotional films than neutral films. Moreover, the number of emotional films recalled was highly correlated with the glucose metabolic rate of the right amygdala during encoding. In contrast, the activity of the amygdala during encoding did not correlate with the recall of neutral films. Alkire et al. (1998) also found that amygdala glucose metabolism did not correlate with the recall of non-arousing stimuli. Overall, emotionally arousing stimuli are remembered better than neutral stimuli (Aldolphs et al., 1997; Cahill et al., 1996; Hamann et al., 1997), and the memory enhancement for emotional events is significantly correlated with amygdala activity during encoding (Cahill et al., 1996).

Amygdala Damage Attenuates the Enhanced Recall of Emotionally Arousing Stimuli

The amygdala supports the enhancement of declarative memory for emotional events (Cahill, 2000; McGaugh, 2004). With selective, bilateral amygdala damage, patients SM and BP provide a relatable example of the normal emotional enhancement provided by the amygdala (Cahill et al., 1995; Aldolphs et al., 1997). Notably, neither SM nor BP had damage to their hippocampi. SM, BP, and controls were presented with a twelve slides accompanied by a narrative. Again, participants were told to pay attention to the slides, and rated the amount of emotion evoked by each slide. Participants were given a multiple-choice test twenty-four hours after viewing the stimuli. Healthy controls and controls with brain damage that did not extend to

the amygdala demonstrated enhanced recall for the slide rated as the most emotionally arousing. Although SM and BP rated this slide as emotionally arousing as the controls, neither SM nor BP displayed enhanced recall of the most emotionally-arousing slide (Aldolphs et al., 1997). Patients SM and BP lack the memory enhancement for emotional material possessed by individuals with intact amygdala. Enhanced recall of emotionally arousing stimuli is attenuated in individuals with bilateral amygdala lesions; therefore, the amygdala supports enhanced recall of emotionally arousing stimuli.

Basolateral Complex of the Amygdala Modulates Declarative Memory for Arousing Stimuli

The amygdala is a heterogeneous structure, and one region, the basolateral complex, plays an especially important role in modulation of declarative memory (McGaugh, 2004). Lesions of the basolateral complex of the amygdala result in alterations to declarative memory for highly arousing tasks, such as, inhibitory avoidance and water maze tasks (Tomaz ,1992 ; Roozendaal and McGaugh, 1996). In fact, many neurotransmitters and hormones exert their effects on memory only if the basolateral complex of the amygdala is intact (McGaugh, 2004). Selective excitotoxic lesions of the basolateral complex of the amygdala block the memory-impairing effects of benzodiazepines on the retention of an inhibitory avoidance task (Tomaz,1992). Additionally, a selective lesion to the basolateral complex of the amygdala also blocks the memory-enhancing effects of post-training systemic injections of dexamethasone, a synthetic glucocorticoid, on the retention of an inhibitory avoidance task (Roozendaal & McGaugh, 1996). Thus, the basolateral complex of the amygdala plays a fundamental role in modulating declarative memory for arousing events.

Much research has directly probed the functions of the basolateral complex of the amygdala through infusions of pharmacological agents in order to manipulate declarative memory. Post-training infusions of norepinephrine, a β-adrenergic receptor agonist, into the basolateral complex of the amygdala enhance declarative memory for a spatial water maze task (Hatfield & McGaugh, 1999). Conversely, post-training infusions of propranolol, a β-adrenergic receptor antagonist, into the basolateral complex of the amygdala impair declarative memory for a spatial water maze task (Hatfield & McGaugh, 1999). Post-training intra-amygdala infusions of flumazenil, which is a benzodiazepine antagonist, enhance memory only when selectively administered to the basolateral nucleus for an inhibitory avoidance task (Da Cunha et al., 1999). Post-training infusions of lidocaine into the basolateral nucleus impairs declarative memory for an inhibitory avoidance task (Parent & McGaugh, 1994). Although manipulations of the basolateral complex of the amygdala can impair or enhance declarative memory for arousing events, there is a clear relationship between emotional arousal or amygdala activation and memory: moderate levels of arousal or amygdala activation lead to enhanced memory, whereas, very high levels of arousal or amygdala activation lead to impaired memory (Gold et al., 1975; Cahill & McGaugh, 1990).

Basolateral Complex of the Amygdala Modulates Declarative Memory for Non-Arousing Stimuli

Although there are many findings that support the role of the basolateral complex of the amygdala in highly arousing tasks, few studies have looked at the basolateral complex of the amygdala's role for declarative memory of neutral or mildly arousing tasks. For example, few studies in this area have used a novel object recognition task, presumably a minimally–arousing task. However, a few studies have explored the relationship between activation of the amygdala

and object recognition memory, including a recent study that infused norepinephrine into the basolateral complex while rats were presented with new objects to remember (Roozendaal et al., 2008). Normally three minutes of an object exposure is not enough time for rats to remember an object twenty-four hours later; however, infusions of norepinephrine into the basolateral complex of the amygdala enabled rats to remember objects one day later (Roozendaal et al., 2008). These recent studies indicate that the basolateral complex of the amygdala can modulate declarative memory even for neutral or mildly arousing events.

Memory Consolidation

The procedure employed by Roozendaal et al. (2008) used post-encoding amygdala manipulations like many of studies described above (i.e., Da Cunha et al., 1999; Hatfield & McGaugh, 1999; Parent & McGaugh, 1994; Roozendaal & McGaugh, 1996) Post-encoding manipulations are those that occur only after the initial study session has ended. The observation that these post-encoding manipulations can enhance (or impair under other conditions) subsequent memory suggests that the amygdala is involved in modulating molecular and cellular processes, thought to be occurring in the hippocampus, in which recently-encoded information is stabilized into a durable memory. The ongoing processes which occur after encoding and stabilize memory traces are called memory consolidation (Dudai, 2005). Immediately after training, memory traces are incomplete; thus, post-encoding manipulations can modulate the memory trace. Memory consolidation enables a pliable, transient memory to be converted into a long-term memory. The long-term memory is a more stable form of the original memory and requires cellular changes, such as, protein synthesis, in the neurons that support a specific memory.

My Honors Thesis Experiment

The goal of our project was to better understand how the amygdala modulates hippocampal-dependent, declarative memory. Previous studies have successfully modulated declarative memory through electrical and pharmacological stimulation; however, these paradigms involved prolonged activation of the amygdala for the entire training session. The present study addresses this issue by refining a method of very briefly electrically stimulating the amygdala with precise timing in hopes of targeting the neural connection between the basal nucleus of the basolateral complex of the amygdala and the hippocampus at only the moments in which the to-be-enhanced objects are encountered by the rats. We wanted to know if moderate levels of electrical stimulation of the basal nucleus could preferentially enhance the declarative memory for specific events within a training session of a novel object recognition task, known to depend on the hippocampus (Clark, Zola, & Squire, 2000). The overall hypothesis was that the amygdala-hippocampal connectivity would be activated by this electrical stimulation, resulting in enhancement of the hippocampal memory processes during encoding of objects. In addition, based on previous demonstrations that amygdala activation at least in part targets prolonged window of memory consolidation (Roozendaal et al., 2008), we expected that the benefit of amygdala stimulation for object memory might be best observed at the longer, one-day, study-test interval.

Method

Subjects

Five male Long Evans rats (350 – 450 g at time of surgery) were housed individually. During the surgical recovery period, rats were given free access to food and water; however, during training and testing, rats were given free access to water but were maintained on a mildly-

restricted food diet so that rats would complete laps for a small food reward. A rats' motivational level to run laps determined the amount of food that he received. Rats were weighed weekly to ensure that they maintained at least ninety percent of their free food weight. Emory IACUC approval was obtained for all procedures.

Surgical Procedure

Surgery was performed to bilaterally implant stimulating electrodes into the basolateral complex of the amygdala. The stimulating electrodes were affixed to the rats' skulls for chronic stimulation experiments. One to three percent isoflurane was delivered to rats by a precision vaporizer. Rats were also given subcutaneous injections of buprenorphine (0.05 mg/kg) prior to surgery to relieve pain and reduce the amount of isoflurane required to maintain sufficient anesthesia. Sufficient anesthesia was confirmed by a lack of response to a toe pinch. Once rats no longer responded to a toe pinch, their skulls were positioned in a stereotaxic frame. Twisted, bipolar stimulating electrodes were implanted in the basal nucleus of the basolateral complex of the amygdala at the stereotaxic coordinates (from Bregma): anteroposterior, -3.7mm; mediolateral, $\pm$ 5.2 mm; dorsoventral, -9.2mm.

Training

Rats were trained to complete laps around a circular track prior to surgery (See Figure 1). After surgery and a week of rest, the rats resumed a mildly-restricted food diet and training on the circular track. Rats were exposed to a few junk objects prior to testing; however, rats were not exposed to the test objects prior to the testing session. All rats completed one hundred and twenty laps on consecutive days prior to testing.

Testing apparatus

Figure 1 illustrates the circular track used to test the rats. The outside diameter of the circular track was 91.4 cm. The track width was 7.6 cm. The circular track had a central arm and three additional platforms on which objects could be placed. The platforms were attached to the outer circumference of the track such that objects could be positioned on the platforms without obstructing a rat's path. Additionally, the objects could be investigated without rats altering their path. The circular track was cleaned with 100% ethanol each time a rat completed his testing for the day.

Objects

During testing, rats were presented with novel junk objects on the object platforms and were allowed to freely explore the objects. A replica of each object was used for subsequent presentations to avoid scent marking. Objects were made of plastic, wood, and plaster and ranged in size from 3cm X 4cm X 1cm to 10cm X 20cm X 10cm. A BLOCK consisted of similarly sized objects, but within a BLOCK, objects were randomly assigned to experimental conditions. All replicas were cleaned with fifty percent ethanol after each use of an object from the collection. The precautions were taken to reduce confounds such as object preference or location preference. For example, the STIM objects for one rat were the OLD objects for the other rat and vice versa, and the rats encountered the three object types equally among the three platforms.

Novel Object Recognition Task

Operational definition of memory. Rats have an innate preference for novelty; therefore, rats spend more time investigating novel and forgotten objects than familiar objects (Ennaceur & Delacour, 1988; Mumby et al., 2002; Manns & Eichenbaum, 2009). This measure has been

validated by other studies in monkeys (Bachevalier et al., 1993; Jutras and Buffalo, 2010) and humans (Fantz, 1964; Diamond, 1990). Investigation of an object was defined as a rat orienting toward an object and whisking. During the novel object recognition task, rats freely explored objects as they completed laps on the circular track for a small food reward. Notably, the reward was not contingent on investigating any object.

Blocks of the novel object recognition task. The objection recognition task consisted of twenty-four BLOCKS. Each BLOCK was comprised of a STUDY phase and either an IMMEDIATE TEST or a ONE DAY TEST (See Figure 2).

Block exclusion criteria. Blocks were excluded from analysis if a rat chewed on an object during any phase. Blocks were also removed if rats skipped an object during the STUDY phase because the results on the TEST phase are interpretable if rat has not previously investigated an object. BLOCKS were not removed if a rat chooses not to investigate an object during either the IMMEDIATE TEST or the ONE DAY TEST.

Phases of the novel object recognition task. The novel object recognition task had two phases: a STUDY phase and a TEST phase. During the STUDY phase, rats encountered objects on the circular track for the first time. The STUDY phase included an inter-trial blank lap and three consecutive laps in which the rat encountered a STIM, OLD, and NEW object (See Figure 2). The order of the object group was randomly assigned and counterbalanced throughout the test session. Electrical amygdala stimulation was administered only during the STUDY phase immediately following exploration of the STIM object (See Figure 2).

There were two versions of the TEST phase: the IMMEDIATE TEST and the ONE DAY TEST. The IMMEDIATE TEST and the ONE DAY TEST only differed in the duration of time that elapsed between the STUDY phase and the particular version of the TEST phase. The

IMMEDIATE TEST began immediately following the STUDY, approximately one hour after the STUDY began. Approximately twenty-two to twenty-six hours elapsed between the STUDY phase and the ONE DAY TEST. The IMMEDIATE TEST was comprised of half of BLOCKS from the STUDY phase, and the ONE DAY TEST was comprised of the other half of BLOCKS. The TEST phases included an inter-trail blank lap and a single lap in which all three objects were presented in the same location as encountered during the STUDY.

Three groups of object conditions. Rats encountered a unique set of three objects during each BLOCK: STIM, NEW, and OLD (See Figure 2). Objects were counter-balanced between rats and placed in the same orientation on the same platform during the STUDY and the TEST.

STIM group. The STIM group contained objects that were paired with electrical amygdala stimulation. During the STUDY, immediately after the rat stopped investigating an object from the STIM group, a rat received brief electrical amygdala stimulation. For either of the two versions of the TEST phase, rats encountered a replica of the STIM object.

OLD group. The rats encountered the object from the OLD group for the first time during the STUDY phase and encountered a replica during one of the two versions of the TEST phase.

NEW group. The NEW group contained objects which were novel in both the STUDY phase and the TEST phase; consequently, each BLOCK included two NEW objects.

Histology

Immediately prior to euthanizing the rats, a 20 μA current was passed through both stimulating electrodes for fifteen seconds to create a lesion in order to confirm the electrode tip placement. Rats were then perfused with formalin. Their brains were sectioned into 35 μm sections, and subsequently, stained with cresyl violet or acetylcholinesterase stains.

Obtaining Data

Each testing session was video recorded. Frames were captured every thirtieth of a second. The time was recorded for each onset and offset of a rat's investigation of an object. The total duration a rat investigated each of the objects was used for data analyses.

Results

I hypothesized that brief electrical stimulations to the basal nucleus of the basolateral complex of the amygdala immediately following object exploration would enhance the declarative memory of an object paired with stimulation and might do so in a way that preferentially benefitted object memory when tested after a relatively long study-test delay. In the novel object recognition task, increased declarative memory is demonstrated by preferential exploration of a novel object over a familiar object (Ennaceur & Delacour, 1988). During the novel object recognition task, rats investigated repeated objects less than new objects. On some laps, rats did not investigate a particular object, and on other laps, rats investigated an object for more than ten seconds. The distributions of the investigation times were significantly skewed (mean: 1.42, range: -0.34 to 3.19); therefore, it was appropriate to use the median of each rat as the measure of central tendency. The distribution of the medians was normal as indicated by the lack of significant values on a Shapiro-Wilk test of normality; therefore, parametric tests would be appropriate to analyze the medians. Figure 3 shows the average of the medians of the absolute time of object investigation for each object condition (STIM, black; OLD, grey; NEW, striped) during the STUDY, IMMEDIATE TEST, and ONE DAY TEST. The results for each portion of the experiment (STUDY, IMMEDIATE TEST, and ONE DAY TEST) are described in more detail below.

During the STUDY, there were no differences between the investigation times of the three object types. Thus, the objects were randomly assigned to different conditions. Moreover, this finding supports that stimulation occurred post-investigation and did not alter the preference for objects types during the STUDY. During the IMMEDIATE TEST, rats investigated STIM and OLD objects significantly less than the NEW objects (paired-samples t test: $t_{(4)} = 2.690$, p=0.05,two-tailed; paired-samples t test: $t_{(4)} = 3.538$, $p<0.05$, two-tailed respectively); therefore, we interpret this as rats had memory for both the STIM and OLD objects (Ennaceur and Delacour, 1988). On the ONE DAY TEST, STIM objects were investigated significantly less than the NEW objects (paired-samples t test: $t_{(4)} = 3.325$, $p<.05$, two-tailed); therefore, interpreted this as rats remembered STIM objects (Roozendaal et al., 2008). Notably, there was no difference in investigation of OLD and NEW objects during the ONE DAY TEST; thus, we interpret this finding as rats did not remember OLD objects after a one day retention period.

A novel object discrimination index assesses memory in novel object recognition tasks by normalizing the data so that the investigation time of the novel object is a percentage of the total investigation time of the novel and familiar object. The novel object discrimination index was calculated for each rat as the ratio of the average of the median investigation times for NEW objects over the sum of the medians of NEW objects and either STIM or OLD objects. Figure 4 shows the average novel object discrimination index score for both STIM and OLD object conditions during the IMMDIATE TEST and ONE DAY TEST. The novel object discrimination score is significantly different than 50% for STIM and OLD objects during the IMMEDIATE TEST (one-sample t test: $t_{(4)} = 3.239$, $p<0.05$, two-tailed; one-sample t test: $t_{(4)} = 5.924$, $p<0.01$, two-tailed respectively). Only the novel object discrimination index for STIM objects during the ONE DAY TEST was significant (one-sample t test: $t_{(4)} = 3.460$, $p<0.05$, two-tailed). Notably,

the novel object discrimination index for OLD objects was not significant on the ONE DAY TEST.

$$\text{STIM vs Novel Object Discrimination Index} = \frac{\text{NEW}}{\text{NEW} + \text{STIM}}$$

$$\text{OLD vs Novel Object Discrimination Index} = \frac{\text{NEW}}{\text{NEW} + \text{OLD}}$$

Discussion

The main finding of the present study was that brief electrical stimulation of the basolateral amygdala immediately following rats' initial encounters with objects enhanced memory for those objects when memory was tested one day later but not when tested approximately one hour later. These results suggest that electrical stimulation of the basolateral complex modulates memory not immediately, but instead during the prolonged period in which a new memory becomes stabilized in the brain, a process called memory consolidation (Dudai, 2004).

Our findings replicate those of Roozendaal et al. (1998) in that rats remember objects one hour but not twenty-four hours after training without the memory enhancement provided by activation of the basolateral complex of the amygdala. The present results add to the previous study in that amygdala activation immediately follows each object exploration to be paired with stimulation. In previous studies, a pharmacological manipulation occurred following the entire testing session (i.e., Da Cunha et al., 1999; Hatfield & McGaugh, 1999; Parent & McGaugh, 1994; Roozendaal et al., 2008; Roozendaal & McGaugh, 1996), so object encounters and amygdala activation were not intimately synchronized. Moreover, in the present study, some objects are paired with amygdala activation, and other objects are not paired with amygdala

activation within a single training session. This differs from previous studies in which pharmacological activation affected all of the object encounters during a training session.

The observation that rats investigated objects paired with stimulation less than objects not paired with stimulation on the one-day test might suggest that the observed effect resembled fear conditioning, as the amygdala has often been implicated in fear learning (Davis, 2000). However, the data from the one-hour test session do not suggest that rats were afraid of objects paired with stimulation. Specifically, during the immediate test there was no difference between the investigation times of the objects paired with stimulation and objects that were not previously paired with stimulation. If rats had acquired fear for the objects paired with stimulation, one would have expected the rats to avoid these objects more so than objects not paired with stimulation, since our paradigm utilizes a preference test. Thus, the reduction in exploration for objects paired with stimulation during the one-day test is better attributed to memory than fear because it is highly unlikely that rats were not afraid of objects paired with stimulation during the immediate test but then afraid of these objects during the one-day test. Furthermore, typical signs of distress in rats during the novel object recognition task were not exhibited; rats did not urinate or defecate excessively and that rats readily completed laps on the track.

The observation that electrical stimulation of the basolateral amygdala enhanced memory apparently with no overt emotional arousal is important to note because others have already found that arousing events and amygdala manipulations modulate declarative memory (Gold et al., 1975; Quirarte et al., 1998). Our findings, affirming those of Roozendaal et al. (2008), indicate that electrical stimulation of the basolateral complex of the amygdala is sufficient to modulate declarative memory even during a minimally arousing task. Since our paradigm selectively activates the basal nucleus of the amygdala following object exploration, our data

support that the putative amygdala modulation of hippocampal memory occurs without the requirement of the extended brain activation that is presumed to underlie a true emotional event.

Our present findings are consistent with previous findings that the amygdala, more specifically the basolateral complex of the amygdala, modulates declarative memory (McGaugh, 2004). Importantly, the present results are the first to show that very brief activation of the amygdala can enhance memory for specific events (encounters with objects paired with stimulation) while leaving unaffected other events (encounters with objects not paired with stimulation) during the same testing session. Our future studies will utilize this paradigm's time specificity of amygdala stimulation in conjunction with hippocampal pyramidal cell recordings. As previously mentioned, the hippocampus is the storage site of declarative memories, such as those formed for the objects during a novel object recognition task (Clark et al., 2000). Simultaneous amygdala stimulation and hippocampal pyramidal cell recordings during the study phase could reveal how the neuronal firing patterns of the objects paired with stimulation differ from the neuronal firing patterns of the objects not paired with stimulation during encoding. Additionally, hippocampal pyramidal cell recordings during the test phases could demonstrate the changes that occurred during memory consolidation. For example, pyramidal cell recordings could depict any differences between the neuronal firing patterns during recognition of objects paired with stimulation and those not paired with stimulation, and such recordings may demonstrate these differences even though there are no measureable differences during the immediate test in the present study. Recordings could also elucidate any differences between the recognition of objects paired with stimulation on the immediate test and objects paired with stimulation on the one-day test.

Our project attempts to fill a gap in the vast but incomplete knowledge of normal declarative memory formation. Our study is imperative as knowledge on the workings of normal memory is vital to the development of effective treatments and cures for memory disorders. Patients with combat-related, post-traumatic stress disorder have exhibited decreased hippocampal volume as compared to controls that were also in combat but did not have post-traumatic stress disorder (Bremner et al., 1995). Additionally, Kim et al. (2005) indicated that pre-training and not immediately post-training amygdala lesions prevent the impairment on hippocampal-dependent memory due to excessive stress and suggest that a deficiency in stress-induced memory impairments may cause post-traumatic stress disorder. According to these data, there is likely impairment in the neural connections between the amygdala and hippocampus. Future simultaneous amygdala stimulation and hippocampal pyramidal cell recordings may have clinical implications that could contribute to the development of novel treatments for post-traumatic stress disorder.

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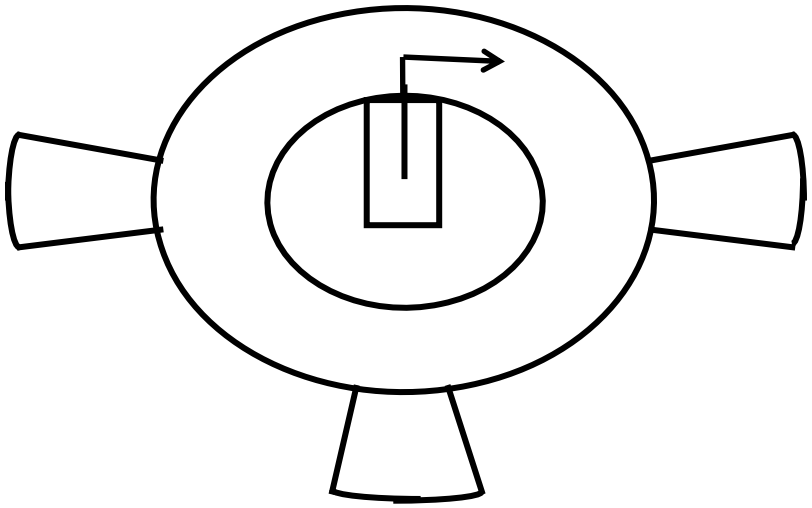

Figure 1. Schematic of track and platform locations.

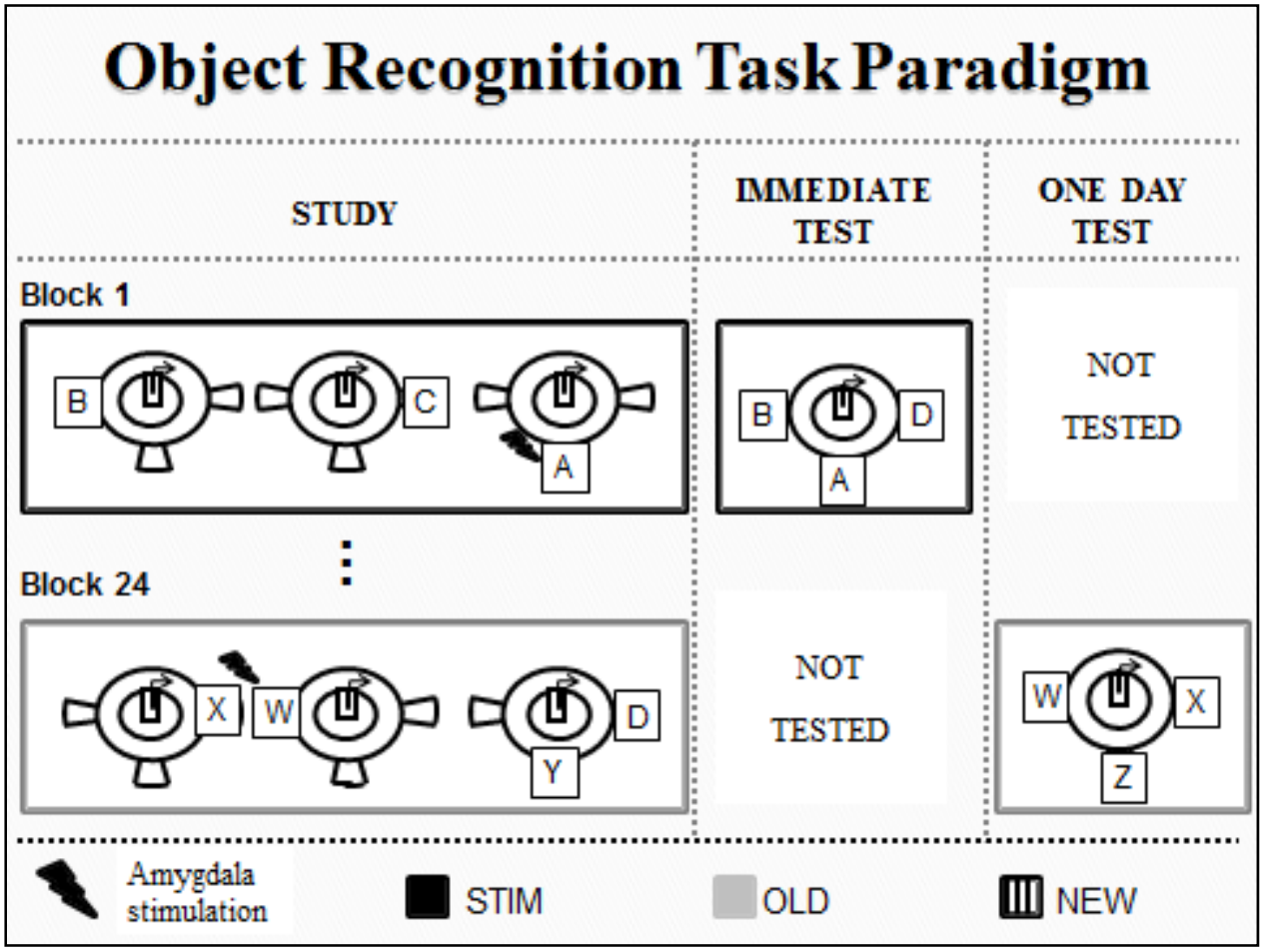

Figure 2. Schematic of the Novel Object Recognition Task Paradigm. Twenty-four BLOCKS of trials were completed over the course of the testing session. Each BLOCK was compromised of a STUDY phase and one of the two versions of a test phase (IMMEDIATE TEST or ONE DAY TEST). During the STUDY phase, rats encountered a STIM, an OLD, and a NEW object. During the TEST phase, rats encountered a replica of the STIM, a replica of the OLD, and a different NEW object.

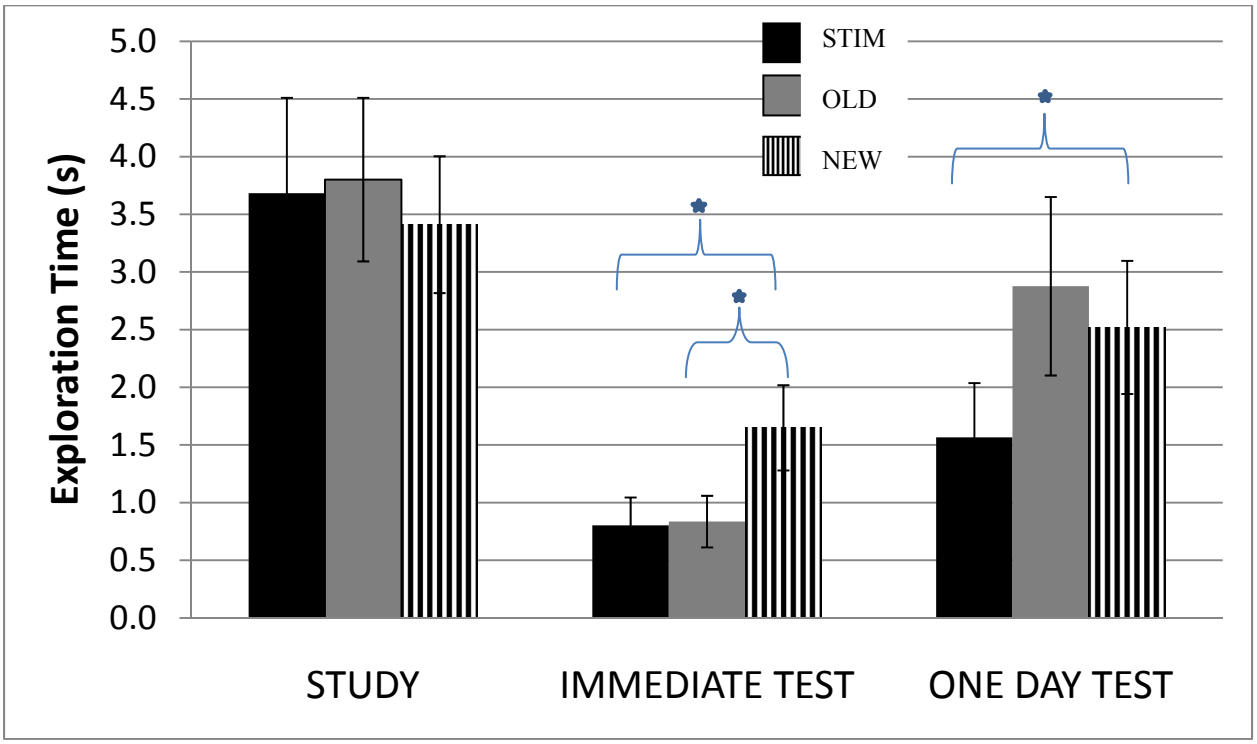

Figure 3. Mean of the Medians for Absolute Time of Object Investigation (n=5). There were no differences in object investigation between object types during the STUDY. During the IMMEDIATE TEST, rats investigated STIM and OLD objects less than NEW objects. This pattern of behavior indicates that rats remember the STIM and OLD objects from the previous encounter with replicas of these objects during the STUDY. Additionally, rats investigate the Objects paired with stimulation for less time than the NEW objects during the ONE DAY TEST.

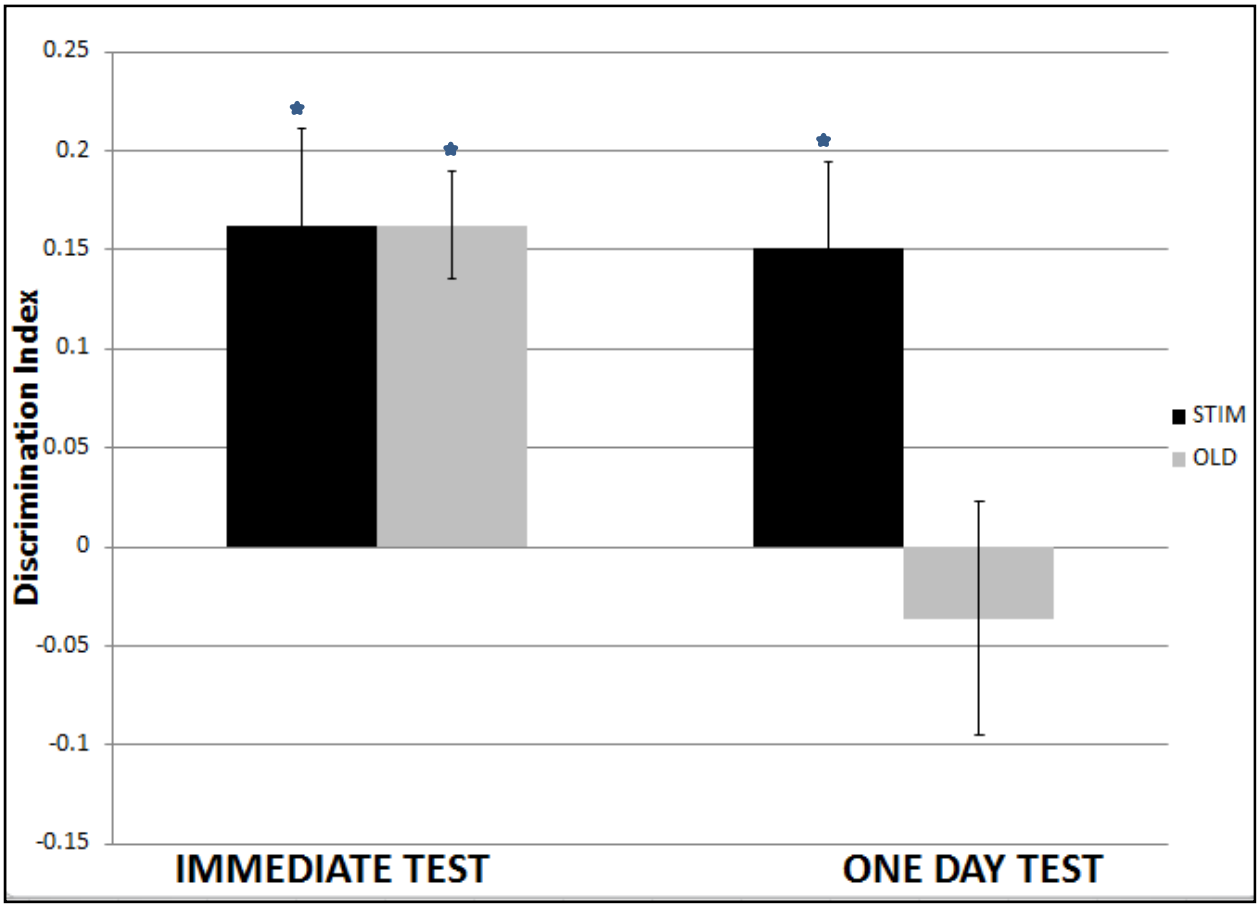

Figure 4. Mean of the Novel Object Discrimination Index Scores (n=5). As expected the rats could discriminate NEW objects from the STIM and OLD objects on the IMMEDIATE TEST. The rats also could discriminate the NEW objects from the STIM objects during the ONE DAY TEST as expected. Notably, the rats could not discriminate the NEW objects from the OLD objects on the ONE DAY TEST.