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An abstract of
A thesis submitted to the Faculty of Emory College of Arts and Sciences
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Abstract

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One prominent theory of hippocampal function is that it is involved in relational memory, or the binding of multiple distinct elements into a coherent representation in memory. Here, we investigated the role of the hippocampus in relational memory by examining eye movements. Across three experiments, four rhesus monkeys viewed naturalistic, complex scenes that were novel, repeated, or manipulated. In the manipulated scenes, a change was introduced in one item which changed in its location or its replacement with a new item. In Experiment 1, normal monkeys made fewer fixations when scenes were repeated without a manipulation and spent more time looking at the altered region in manipulated scenes. Consistent with previous studies in humans, the monkeys' eye movements differed depending on the viewing history of the scene suggesting successful memory formation. In Experiment 2, a hippocampal-lesioned monkey displayed deficits in this task that revealed a significant lesion by manipulation type interaction. This is consistent with findings from human amnesic patients and suggests that the hippocampus is critical for relational memory. In Experiment 3, we studied the role of context in relational memories. Three normal and one hippocampal-lesioned monkey viewed scenes with and without a background. We found that control monkeys displayed better memory when the scenes were presented in a context, i.e., with a background. Interestingly, the hippocampal-lesioned monkey demonstrated significant relational memory, only when the scenes were presented without a background. These data suggest that the presence of a background context normally acts as an aid to relational memory formation, but this requires an intact hippocampus.

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INTRODUCTION

Declarative Memory and Eye Movements as a Measurement of Memory

The critical participation of the medial temporal lobe (MTL), and especially the hippocampus, in memory has been well established since the earliest reports of profound amnesia following MTL resection in the patient H.M. (Scoville and Milner, 1957). Amnesic patients were unable to form new memories (anterograde amnesia) and retrieve certain recently-acquired memories (retrograde amnesia) (Scoville and Milner, 1957). Since that seminal report, attempts to characterize its role with more precision have been undertaken by many researchers, and a variety of different accounts have emerged. Many studies investigating both human amnesic patients and animal models of amnesia have contributed to our understanding of the function and MTL structures, including the hippocampus, entorhinal, perirhinal and parahippocampal cortices, to learning and memory (Squire and Zola-Morgan, 1991).

One major form of memory, declarative memory, supports the ability to remember facts and events and depends on the integrity of the hippocampus and related structures. This type of memory is compromised following damage to the medial temporal lobe, including the hippocampus and the surrounding cortical areas (Squire and Zola-Morgan 1991). Declarative memory for events, semantic information, and objects can be expressed and reported verbally (by humans) or non-verbally through responses such as eye movements, gestures and other behavioral responses (by both humans and animal models) (Purves et al., 2008). Impairment of this form of memory is an important component of diseases such as Alzheimer’s disease, schizophrenia, temporal lobe epilepsy, and depression, and this impairment can have a severe impact on the quality of life of these patients. Despite extensive research, we still have an incomplete understanding of the neuronal mechanisms underlying declarative memory formation and retrieval.

Eye movements have been demonstrated to be a useful way to assess memory for complex images (Ryan et al., 2000; Smith et al., 2006). Because of an innate preference for novelty, human subjects make fewer eye fixations on objects and regions that they have already previously viewed in comparison to novel items (Althoff and Cohen, 1999). Eye movements have also been utilized in learning and memory studies with monkeys in our laboratory (Jutras and Buffalo, 2010; Jutras, Fries and Buffalo, 2009). Specifically, these studies have involved not only tasks with correct and incorrect answers warranting reward for the monkeys (for example, delay match to sample), but also involved free viewing tasks in which subjects viewed stimuli without anticipation of reward. For example, normal human subjects and monkeys showed memory by preferentially looking at the novel object in the visual paired-comparison task (Overman et al., 1993; Nemanic et al., 2004). Although differences in memory encoding techniques have been noted for differences in these two types of tasks (Nemanic et al., 2004), whether passive learning or active learning, memory can still be assessed through familiarity and event recollection in a free viewing task.

Relational Memory

One prominent theory of hippocampal function is that it is particularly involved in relational or configural learning, the binding of representations of multiple distinct elements into a coherent representation in memory, including spatial, sequential (temporal), and associative (co-occurrence) relations among items (Konkel et al., 2008).

One way of assessing this type of memory is by manipulating an image slightly and assessing whether or not a subject looks at a specific region of this image when it is repeated, which we named the Scene Manipulation Task (SCM). It has been shown that declarative memory is involved in the recognition of an image that has been manipulated (Ryan et al., 2000). Normal human subjects spent more time looking at a region of repeated visual scene that had been altered in comparison to novel scenes and repeated scenes without manipulation. In contrast, amnesic patients with hippocampal damage did not demonstrate this behavior, and their performance was interpreted as revealing a deficit in relational memory (Smith et al., 2006). Thus, the hippocampus is critical in successful relational memory formation (Eichenbaum et al., 1994). Importantly, it was shown that they preferentially viewed the altered region even when normal human subjects were not directed to look at the region of the manipulation and had no expectation that memory was being tested (Smith et al., 2008; Smith et al., 2006). These data suggest that this behavioral task might be useful for assessing relational memory in monkeys.

In order to fully understand how relational memories are encoded, it is necessary to further explore item and context relationship. Recent work has begun to illuminate how the different elements of an experience — the objects and the context — are encoded within the medial temporal lobe (Davachi, 2006). This association between an item and its background context has been labeled as “contextual binding” (Chalfonte and Johnson, 1996). Studies have shown differential functions of the hippocampus versus surrounding MTL cortices, for relational memory versus item memory respectively (Cohen et al., 1997; Smith and Mizumori, 2006), but their exact roles and their interactions remain underspecified. Explicitly, these findings demonstrate that the perirhinal cortex encodes memory for individual items, whereas the level of hippocampal processing correlates with relational memory and recovery of episodic details. Furthermore, functional magnetic resonance imaging evidence in humans suggests that MTL input structures, the perirhinal and posterior parahippocampal cortices, participate in the encoding of objects and their context, providing different inputs to the hippocampus (Davachi, 2006; Smith and Mizumori, 2006).

However, there still exists considerable debate as to whether the hippocampus and the surrounding MTL areas share similar roles in the binding of context, spatial information and item, nonspatial information (Ross and Slotnick, 2008; Susanne et al., 2004). Specific subregions in the MTL are differentially associated with item memory and memory for context (Kohler et al., 2002). These subregions of the MTL have also been shown to contribute interactively to spatial and object memory processing (Bachevalier and Nemanic, 2008). For example, the parahippocampal cortex has been shown to be important for processing of item information while spatial information may be preferentially processed in the entorhinal cortex and the hippocampus (Hayes et al., 2007; Kohler et al., 2005). Buckmaster et al. also showed that the entorhinal cortex is necessary for relational organization of memory in monkeys (2004).

These findings not only suggest that the hippocampus mediates the encoding of relational memories in an object to background compound, but also suggest that the other MTL structures mediate the encoding of either the object or the background which cannot be responded to dependent on one or the other. For example, a parahippocampal-lesioned subject presented with a familiar object on a new background would perceive it as a new stimulus. Additionally, monkeys with hippocampal damage are more severely impaired in object-in-place discrimination trials than on either object discrimination or place discrimination trials alone (Gaffan, 1994) in addition to performance on the free-viewing visual paired comparison task (Pascalis et al., 2009). Thus, the SCM task provides a great way of measuring relational memories between not only the item to item, but also the item to context relation. However, there have yet to be studies focusing on the context portion in relation to item manipulations within a complex scene like in the scene manipulation task.

Using Rhesus macaques as a Model for Relational Memory

We selected rhesus macaques (*Macaca mulatta*) as our animal model because they have demonstrated a capacity for completing a task that involves selectively fixating on and remembering complex visual images (Buffalo et al., 2009; Jutras and Buffalo, 2010; Overman et al., 1993; Nemanic et al., 2004). Monkeys have also been used effectively in studies that measured memory in other MTL-dependent tasks such as object matching, discrimination learning, object discrimination and spatial delayed response (Zola-Morgan and Squire, 1985**).** In addition, monkeys have also been used in various lesion studies regarding long term memory tasks, including spatial and relational memories (Lavenex et al., 2006; Zola et al., 2000; Bachevalier and Nemanic). However, hippocampal lesioned monkeys have not previously been used to assess relational memory in the Scene Manipulation Task.

Goals of Current Study

There were three main goals of this project. The first goal was to replicate the findings of Smith et al. (2008) with Rhesus macaques to examine whether they have the capability to demonstrate relational memory formation in a similar task. Smith et al.'s results showed that humans displayed a preference for looking at a manipulated region of a repeated scene (or stimulus) in comparison to that same region in novel scenes and repeated scenes without manipulation. Here, we hypothesized that control Rhesus macaques would demonstrate this same performance and would preferentially view manipulated regions of complex visual scenes, indicating successful relational memory formation.

The second goal was to determine whether the hippocampus is necessary for successful relational memory formation in monkeys. Here, we hypothesized that the hippocampus is necessary for this type of relational memory task and a monkey with a selective lesion of the hippocampus would display impairment in relational memory formation.

The third goal of this study was to examine the role of context in relational memory formation. Based on the current understanding of the role of the hippocampus in relational binding of item to context, we hypothesized that items in scenes with a background context would be remembered better than items in context-free scenes in normal macaque monkeys, and that the hippocampus would be required only for remembering items within a context.

METHODS

Subjects

The subjects (JN, IW, MP, and TT) were four male rhesus macaques (*Macaca mulatta*), between 8 and 10 years old, with experience and training in various other eye movement dependent memory tasks (such as the Visual Preferential Looking Task and Delayed Match to Sample) that involved digital photographic images presented on a computer screen. The monkeys lived individually in protected contact housing that permitted social contact with adjacent cage mates through a partition while physically separating them to maintain the integrity of their surgically implanted head posts and chambers.

All monkeys were on a controlled food diet under guidelines from the Emory University Institutional Animal Care and use Committee and Yerkes National Primate Research Center to provide motivation for food rewards. The food reward consisted of a special chow mixture made from Lab *Diet*® monkey biscuits, applesauce, and water blended to a smooth consistency. The chow mixture was administered via a tubing system connected to a metal sipper tube fixed to the chair and easily accessible to the monkey’s mouth. The chow mix was dispensed by a *Masterflex*® pump which was connected to a computerized reward system. The monkeys had been involved in other behavioral tests in our lab and were accustomed to receiving all of their food for the day during the behavioral session. The monkeys were weighed each testing day to ensure that each monkey was receiving enough food and was growing at a healthy rate.

Stimuli

Large, full screen (25^ox35^o) images of pictures taken from PicasaWeb (*Google, Inc.*) and Fickr.com were presented. Some of these images were altered, via Adobe Photoshop, such that an object was moved from the original location or replaced with a different object. Only naturalistic scenes were selected, including those from outdoors and indoors. In the first two experiments, images containing a wide variety of objects were chosen, ranging from three to more than fifteen. Due to evidence that relational information is still intact following MTL damage only with a low memory load (Jeneson et al., 2010), the third experiment only included scenes with eight or more objects. The manipulated objects were either manipulated within the original scene or added into the scene into an empty space. In the replaced manipulation, the new objects added were similar to the original object, i.e. an airplane to a helicopter. Objects within the scene that seemed particularly salient were not chosen as the manipulated object. In addition, we chose objects located somewhere in the middle of the scene, avoiding objects in the foreground or background. For the moved manipulation, the selected object was moved to a different location that didn't have any objects in the original scene.

For the third experiment, low-context scenes were created by selectively cropping all salient objects; those that had a strong contrast in color and had a defined shape (see Figure 10 for an example). A randomly selected single color gradient background was placed behind the cropped items.

Behavioral Testing

Each monkey was trained to sit in a primate chair with an internal touch-sensitive metal bar in a closed room with minimal lighting. Eye movements were captured via an infrared eye tracking system (*ISCAN® Primate Eye Tracking Laboratory, Model RK-826 PCI Pupil/Corneal Reflection Tracking System*) and monkeys were head-fixed in order to assure a steady eye scan. The experimental stimuli were presented using a data acquisition and experimental control program (*NIMH CORTEX*, http://www.cortex.salk.edu/) on a 19 inch CRT monitor running at 120Hz, noninterlaced refresh rate positioned 60cm in front of the monkey.

Each data collection session lasted approximately 35 minutes and consisted of 5 trials of a calibration task followed by 36 trials displaying stimuli interleaved with 5 trials of the same calibration task. The calibration task was a simple response task (color change), in which a 5 by 5 mm grey square appeared on the black screen and after a randomized time between 500 and 1500 ms, the square changed colors to an isoluminant yellow. The monkeys were rewarded when they responded to the color change by releasing their hold on the touch bar within 300 ms of the color change.

The color change task was used prior to the initial scene trial to calibrate the eye scan and then to maintain motivation throughout the rest of each session. Following calibration, the monkeys were tested on the Scene Manipulation Task. Large, full screen (25^{o}x35^{o}) images of pictures were presented. Each trial or group of stimuli included two images, a novel scene followed by the repeated scene, with or without manipulation. Monkeys were not rewarded during the scene trials. Between each scene trial, 5 color change trials were presented in order to motivate the monkeys continue working in the task. The outline for the general structure of the task is provided in Figure 1.

In experiment 1, four normal monkeys viewed both the novel and repeated stimuli until they accumulated 10 seconds of total time looking at the stimuli. One monkey, TT, was used for eight sessions of the SCM task and was also used for experiment 2. The other monkeys were used for 12 sessions each. 36 stimuli were presented for each session. Monkey TT was then prepared with a lesion of the hippocampus. Following surgery and recovery, TT was tested in seven sessions of the SCM task with all novel stimuli. In experiment 3, three of the four normal monkeys from the first experiment and the hippocampal-lesioned monkey from the second experiment viewed the novel stimuli for ten seconds and repeated stimuli for six seconds. Each session included 18 trials of novel low context and 18 trials of novel high context stimuli. These stimuli were repeated one week later in another set of 36 trials, in which the stimuli contained the opposite conditions. For example, if scene A-high context was presented on day 1, scene A-low context was presented at least one week later.

The total amount of trials (stimuli) each monkey viewed that were used in the analyses is represented in Table 1. Trials in which the monkey did not fixate on the CR in the novel presentation were excluded, along with trials in which the animal showed little interest by viewing outside the screen for the majority of the trial.

Surgery

For the hippocampal lesion in monkey TT, the cranium was opened and the dura was opened to allow the needle of a 10 µl Hamilton syringe held by a Kopf electrode manipulator (*David Kopf Instruments, Tujunga, CA*) to be lowered at each injection site where 2.4 µl ibotenic acid (10 mg/mL in PBS, pH 7.4) was injected at a rate of 0.4 µl per minute. Both hemispheres were injected simultaneously and after the completion of each injection, the needle remained in place for three minutes to allow the neurotoxin to diffuse and to minimize any potential for it to spread up the needle track during retraction. The monkey's brain was scanned via a magnetic resonance imaging (MRI) one week after the lesion surgery. The monkey was allowed to recover for one month prior to testing on the SCM task in experiment 2.

Behavioral Analysis

All analyses were performed in Matlab (*Mathworks, Inc.*) with custom programming. To quantify memory, the amount of time the monkey looked at the manipulated region in the manipulated image compared to the same region in repeated images without manipulation was used. For manipulated images, the proportion of fixations in the "critical region" (the regions that contain the manipulation) and the monkey's viewing time in that region were calculated and compared to the controls (novel and repeated scenes without manipulation). Critical regions (CRs) were rectangles drawn on the computer by the experimenter and thus outlined coordinates of the manipulated object within the scene.

In addition, the number of transitions from a fixation outside the critical region to a fixation inside the critical region were measured, along with the duration and the number of fixations as well as when the saccade inside the region occured. For each measure, student's t-tests were used to examine the difference between novel, repeated, and repeated with manipulation conditions. Two-way ANOVAs were performed to examine interactions between hippocampal lesion and scene-type manipulation in experiment 2 and to examine interactions between context and scene-type manipulation in experiment 3.

Additional analyses included a time-course analysis of the proportion of viewing time inside the critical region in which the entire lengths of each trial were separated into bins of 500ms. In experiment 2, a subtraction analysis between condition types was performed to compare each manipulation condition to the lesion. In experiment 3, a correlational analysis was performed between the viewing patterns of the high context scene versus the low context scene in order to confirm that the monkeys fixated on similar objects and that only the most salient objects were cropped out of a scene to make the low context images.

RESULTS

Experiment 1

Four monkeys freely viewed complex, full sized, naturalistic scenes in which an object within the scene may have been manipulated either by being replaced by another, similar object, or by being moved to a different location in the scene. The monkeys displayed strong interest in the scenes, and for the most part, fixated on the multiple items within the scene. However, trials in which the monkey did not fixate on the critical region (CR), i.e., the region that would be manipulated, in the novel presentation were excluded, along with trials in which the animal showed little interest by viewing outside the screen. A total of 1449 scenes were used in the analyses, and of those 479 were repeated without manipulation, 480 were repeated with a replaced item, and 490 were repeated with a moved item (Table 1). Examples of viewing patterns of three different monkeys are shown in Figure 2.

Relational memory formation was defined by an increase the amount of time the monkey looked at the manipulated region in the manipulated image versus the same region in repeated images without manipulation. For manipulated images, the proportion of fixations in the CR and the monkey's viewing time in that region were calculated and compared to the repeated scenes without manipulation. Fixations were defined by the monkeys' saccadic eye movements and a change in velocity and acceleration of the eye movements (Jutrus and Buffalo, 2010). In addition, the number of transitions from a fixation outside the critical region to a fixation inside the critical region were measured, along with the duration and the number of fixations as well as when the saccade inside the region occurs.

When averaged across all trials, normal monkeys displayed a significant decrease in the average proportion of viewing time, proportion of fixations, and transitions into and out of the CR between the novel presentation and the repeated presentation without manipulation (all p values <.01) (Figure 3). This suggests that the monkey's viewing patterns differed between the novel presentation and the repeat presentation in that the monkey may have viewed regions of the scene that it hadn't in the first presentation. T-tests also revealed significant differences between the control presentation (repeated without manipulation) and the two repeated presentations with manipulations (all p values <.01) (Figure 3).

We also found that the monkeys displayed quick appreciation of the manipulations, even within the first two seconds post stimulus onset (Figure 4). To quantify this data, we selected the first three seconds of the trials using the same analyses performed over the full ten seconds. Here, we found the same results, that there was a significant increase in percentage of fixations and viewing time in, and transitions into and out of the CR for the manipulated, compared to repeated but unmanipulated scenes (all p values <.001). By defining these viewing preferences as a measure of memory, we suggest that monkeys demonstrate relational memory in the SCM task.

Experiment 2

In experiment 2, we attempted to identify whether the hippocampus is critical for the behavior seen in experiment 1. In one monkey prepared with a restricted lesion of the hippocampus, post-surgical MRI revealed bilateral hyperintensity specific to the hippocampus, which is consistent with edema that arises from cell death caused by the ibotenic acid (Figure 6). Although proper quantification of cell death can only be examined through histology, the FLAIR images suggest that the damage is specific to the hippocampus and does not affect other structures within the MTL.

One month after the lesion surgery, the monkey was tested on the SCM task in the same paradigm as experiment 1. Novel scenes were used for the lesioned monkey and 221 total trials were used in the analyses (Table 1). After the hippocampal lesion, the monkey failed to show a viewing preference in the CR for scenes with a move manipulation (p=.698) and also did not display a lack of viewing preference in the CR for scenes without a manipulation (p=.722) (Figure 7). Nevertheless, the monkey still exhibited a significant increase in viewing time in the CR for the replaced object manipulation compare to scenes repeated without a manipulation (p=.001). However, 2-Way ANOVAs revealed significant interactions between the manipulations and the lesion (specifically, F(1)=4.47, p=.0307 for interaction between lesion and the repeat without manipulation, F(1)=5.83, p=.017 for interaction between lesion and the replaced object manipulation, F(1)=7.61, p=.006 for interaction between lesion and the moved object manipulation).

In a subtraction analysis between the repeat and novel conditions, replaced and repeat conditions, and moved and repeat conditions, there were significant differences for the amount of change in the proportion of viewing time in the CR before the lesion and after the lesion (all p values <.01) (Figure 8). This suggests that although the monkey still displayed gaze preference for the replaced object manipulation, the lesion has significantly impaired normal relational memory formation. In addition, when examining the proportion of viewing time in the CR throughout the entire ten seconds of the trials, the lesioned monkey did not display appreciation for the manipulated object within two seconds as seen in control monkeys (Figure 9). Together, these data suggest that normal formation of relational memories requires an intact hippocampus.

Experiment 3

In experiment 3, we attempted to identify the extent to which context, or the background in a scene, is necessary for successful relational memory formation. We also examined how the hippocampus may be involved in relational memory formation when the context is absent. The hippocampal-lesioned monkey from experiment 2 and three of the four normal control monkeys from experiment 1 viewed scenes that contained high and low contexts (Figure 10). A total of 296 low context images and 295 high context images were used for analysis for control monkeys while a total of 100 low context images and 98 high context images were used for analysis for the hippocampal-lesioned monkey (Table 1). Scan patterns of low and high context scenes reviewed similar viewing and fixation patterns (Figure 11), indicating that the cropped items used in the low context scenes were indeed salient and did not necessarily belong to the background.

On average, control monkeys displayed similar behavior to that of experiment 1 for both the high context and low context scenes (Figure 12). However, analyses revealed that relational memory performance was enhanced in high context scenes relative to low context scenes. A 2-way ANOVA comparing the proportion of viewing time in the CR between the repeated and replaced conditions, across high and low contexts revealed a significant interaction (F(1)=12.16, p=.0006). Furthermore, a subtraction analysis revealed significant differences in the amount of change in proportion of viewing time in the CR between high context and low context scenes with respect to the replaced and repeat conditions and moved and repeats conditions (specifically, t(98)=3.28, p=.0014 for replaced and repeat conditions, t(96)=2.77, p=.0069 for moved and repeat conditions) (Figure 13). This suggests an enhancement in gaze preference of normal monkeys for manipulated scenes when there is a high context versus when the context is absent.

The hippocampal-lesioned monkey, on the other hand displayed the opposite pattern of performance. Although the lesioned monkey displayed similar impairments in relational memory formation, as shown in experiment 2 when scenes contained a high context, this monkey was demonstrated significant relational memory when scenes lacked a high context (Figure 14). In low-context scenes, this monkey displayed an overall increase in fixations and looking time within the CR for scenes containing a replaced item manipulation (p=.025, p=.022, respectively) or a moved item manipulation (p=.039, p=.044, respectively). In addition, the animal also displayed a quick appreciation of the manipulation of less than 2 seconds post stimulus onset (Figure 15), which was only shown in normal control monkeys (Figure 4). Taken together, these data suggest that the impairment shown by the hippocampus lesioned animal in experiment 2 is not present when scenes lack a strong background context.

DISCUSSION

Monkeys Display Memory for Relational Aspects of Complex Scenes

Consistent with previous human studies (Smith et al., 2006, Smith et al., 2008, and Ryan et al., 2000), the present data suggest that monkeys spend more time viewing manipulated regions of complex scene, both for moved objects and for replaced objects. These data suggest that monkeys form memories for the relational aspects of the scene.

To our knowledge, this is the first study to demonstrate that monkeys have the capability to perform the SCM task and have the capacity for this form of relational memory. Even with a short delay between encoding and recognition phases, these data are consistent with reports that the hippocampus can retain relational memories at short delays in humans (Jeneson et al., 2011; Hannula and Ranganath, 2008). The present data also suggest that monkeys have a very quick appreciation of the manipulation in both the replaced item manipulation as well as the moved item manipulation. This suggests that the monkey notices the change in the scene almost immediately after stimulus onset. However, further analysis of the different components of the stimuli must be examined. For example, the varying number of objects in the scene, the size of the manipulated object, the distance of the moved object in the moved item manipulation, and the subtlety of the new object in the replaced item manipulation were not controlled for in this experiment but could be separated into subsets to analyze behavioral effects of different modalities within each scene.

Hippocampal Lesions Impair Relational Memory Formation

In the second experiment, we found that, following a restricted lesion of the hippocampus, the monkey's eye movements did not reflect past history with the scenes. The monkey did not discriminate between novel and repeated scenes and did not spend more time looking at the altered region for the relational (moved) manipulation. Although the monkey did show increased looking time at the replaced item in the item manipulation, this was significantly lower than pre-operative levels. This may be due to a small memory load in the majority of the scenes, thus causing a difference in the level of difficulty between the replaced item manipulation and the moved item manipulation similar to findings with memory load in humans (Jeneson et al., 2010; Jeneson et al., 2011). The animal may have just been relying on working memory processes, which occurs mostly with low memory loads (Jeneson et al., 2010; Hannula and Ranganath, 2008) and do not require the hippocampus. However, two-way ANOVAs comparing novel vs repeat, repeat vs item manipulation, and repeat vs relational manipulation all demonstrated a significant lesion by scene type. These data are consistent with findings from human amnesic patients (Ryan et al., 2000; Smith et al., 2006) and suggest that the hippocampus is critical for both item and relational memory.

Hippocampal Lesions Impairs Relational Memory Formation in Scenes with High Context but Not Low Context

The results from Experiment 3 suggest that this impairment for successful relational memory formation is not present when the hippocampal-lesioned monkey is presented with scenes without a background. Accordingly, these data suggest that the hippocampus is not needed for item to item relational memories, and that it is only needed for complex, item to context images. In the absence of a hippocampus, the monkey may have adopted a new and different strategy to remember the low context scenes, perhaps by use of other MTL structures such as the parahippocampal cortex. Since previous studies have suggested that these other structures in the MTL are involved in gathering different visual information, whether the context or the item (Hayes et al., 2007; Davachi, 2008; Howard et al., 2011), the hippocampus may not be required to form a memory for the scene without necessary parts (i.e. a scene without a background or without items).

This study is consistent with Pascalis et al.'s finding that hippocampal-lesioned patients are impaired in the visual-paired comparison task only after a change in the background (2009). Further, since the hippocampus is known to bind these separate entities, or item to context, for successful relational memory formation (Bachevalier and Nemanic, 2008), a lack of a high context may have induced the use of only the other MTL structures and not the hippocampus. The context in this case, or the presence of a background in a complex scene, may also be characterized as a spatial component of the scene. If the relational or spatial component is not present, then the memory for the scene may purely be based on item to item memory, for which the animal is not impaired. More specifically, place cells within the hippocampus have been found to be involved in memory for contextual representations within an item-to-context paradigm (Smith and Mizumori, 2006). Without the hippocampus (and thus place cells), an animal may not be able to form contextual representations in a complex scene.

One significant flaw of this study is the limited number of lesioned monkeys. Additionally, further lesion experiments should be performed throughout individual portions of the MTL to examine the specific role of each structure in relation to successful relational memory formation. Scenes lacking items and only containing the background, instead of the vice versa, should also be utilized to understand how item memories are encoded in the MTL.

High Context Yields Better Relational Memory Formation

In contrast to the good performance in low-context scenes observed in the hippocampal-lesioned monkeys, normal monkeys demonstrated better memory for high-context scenes relative to low-context scenes. Although control monkeys were not impaired with the low-context scenes, they displayed less viewing preference for the manipulations when the scenes lacked a high context background than when the scenes contained a naturalistic, high contextual background. To our knowledge, this type of experiment has never been performed in monkeys and suggests an important role for context in relational memory formation. However, it is difficult to clearly identify how context may play a role in the monkey’s perception of a scene. In humans, items in contexts that belong or have meaning to that context that it’s presented in have been shown to be remembered better (Davachi, 2006). In addition, contexts constrain experience, providing a place where things are located (Rudy, 2009), which may aid memory formation. Again, the context, or the background in a complex scene in our case, may introduce a spatial component that may assist memory for the relational aspects of that scene.

Among the cues that can become associated with a memory are cues from the context in which the memory was formed (Smith et al., 1978). In this case, studies have suggested that if at test such contextual cues could be retrieved, the subject would have additional ways to reactivate the target memory. This has been studied extensively in rats, but not in monkeys. Object recognition studies have provided evidence that rodents not only can recognize previously experienced objects, they also know where the object was experienced (Dellu et al. 1997; Dix and Aggleton 1999). For example, rats spent more time investigating the object new to the test context than the object that was experienced previously in that context. This provides evidence that the rat established a representation of the two contexts and remembered the context in which the objects were initially encountered. This is very similar to the findings in our study in that normal monkeys have a higher appreciation of manipulations when a context is present than when the context is absent.

Unfortunately, this experiment cannot imply that the high contextual backgrounds on naturalistic stimuli used in this entire study were “meaningful” in the same way they are to humans. Further studies using human subjects on this task may shed more light on how a high, meaningful context within a visual scene may contribute to successful relational memory formation.

REFERENCES

Althoff, R. R., & Cohen, N. J. (1999). Eye-movement-based memory effect: a reprocessing effect in face perception. J Exp Psychol Learn Mem Cogn, 25(4), 997-1010.

Bachevalier, J., & Nemanic, S. (2008). Memory for spatial location and object-place associations are differently processed by the hippocampal formation, parahippocampal areas TH/TF and perirhinal cortex. Hippocampus, 18(1), 64-80. doi: 10.1002/hipo.20369

Buckmaster, C. A., Eichenbaum, H., Amaral, D. G., Suzuki, W. A., & Rapp, P. R. (2004). Entorhinal cortex lesions disrupt the relational organization of memory in monkeys. Journal of Neuroscience, 24(44), 9811-9825. doi: Doi 10.1523/Jneurosci.1532-04.2004

Buffalo, E. A., Bellgowan, P. S. F., & Martin, A. (2006). Distinct roles for medial temporal lobe structures in memory for objects and their locations. Learning & Memory, 13(5), 638-643. doi: Doi 10.1101/Lm.251906

Chalfonte, B. L., & Johnson, M. K. (1996). Feature memory and binding in young and older adults.

Cohen, N. J., Poldrack, R. A., & Eichenbaum, H. (1997). Memory for items and memory for relations in the procedural/declarative memory framework. Memory, 5(1-2), 131-178. doi: 10.1080/741941149

Cohen, N. J., Ryan, E., Hunt, C., Romine, L., Wszalek, T., & Nash, C. (1999). Hippocampal system and declarative (relational) memory: Summarizing the data from functional neuroimaging studies. Hippocampus, 9(1), 83-98.

Davachi, L. (2006). Item, context and relational episodic encoding in humans. Current Opinion in Neurobiology, 16(6), 693-700. doi: DOI 10.1016/j.conb.2006.10.012

Dellu, F., Fauchey, V., LeMoal, M., & Simon, H. (1997). Extension of a new two-trial memory task in the rat: Influence of environmental context on recognition processes. Neurobiology of Learning and Memory, 67(2), 112-120.

Dix, S. L., & Aggleton, J. P. (1999). Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition. Behavioural Brain Research, 99(2), 191-200.

Gaffan, D. (1994). Scene-Specific Memory for Objects - a Model of Episodic Memory Impairment in Monkeys with Fornix Transection. Journal of Cognitive Neuroscience, 6(4), 305-320.

Hannula, D. E., & Ranganath, C. (2008). Medial temporal lobe activity predicts successful relational memory binding. J Neurosci, 28(1), 116-124. doi: 10.1523/JNEUROSCI.3086-07.2008

Hayes, S. M., Nadel, L., & Ryan, L. (2007). The effect of scene context on episodic object recognition: Parahippocampal cortex mediates memory encoding and retrieval success. Hippocampus, 17(9), 873-889. doi: Doi 10.1002/Hipo.20319

Howard, L. R., Kumaran, D., Olafsdottir, H. F., & Spiers, H. J. (2011). Double Dissociation between Hippocampal and Parahippocampal Responses to Object-Background Context and Scene Novelty. Journal of Neuroscience, 31(14), 5253-5261. doi: Doi 10.1523/Jneurosci.6055-10.2011

Jeneson, A., Mauldin, K. N., Hopkins, R. O., & Squire, L. R. (2011). The role of the hippocampus in retaining relational information across short delays: The importance of memory load. Learning & Memory, 18(5), 301-305. doi: Doi 10.1101/Lm.2010711

Jeneson, A., Mauldin, K. N., & Squire, L. R. (2010). Intact Working Memory for Relational Information after Medial Temporal Lobe Damage. Journal of Neuroscience, 30(41), 13624-13629. doi: Doi 10.1523/Jneurosci.2895-10.2010

Jutras, M. J., & Buffalo, E. A. (2010). Recognition memory signals in the macaque hippocampus. Proc Natl Acad Sci U S A, 107(1), 401-406. doi: 10.1073/pnas.0908378107

Jutras, M. J., Fries, P., & Buffalo, E. A. (2009). Gamma-band synchronization in the macaque hippocampus and memory formation.. J Neurosci, 29(40), 12521-12531. doi: 10.1523/JNEUROSCI.0640-09.2009

Kohler, S., Crane, J., & Milner, B. (2002). Differential contributions of the parahippocampal place area and the anterior hippocampus to human memory for scenes. Hippocampus, 12(6), 718-723. doi: 10.1002/hipo.10077

Kohler, S., Danckert, S., Gati, J. S., & Menon, R. S. (2005). Novelty responses to relational and non-relational information in the hippocampus and the parahippocampal region: a comparison based on event-related fMRI. Hippocampus, 15(6), 763-774. doi: 10.1002/hipo.20098

Konkel, A., Warren, D. E., Duff, M. C., Tranel, D. N., & Cohen, N. J. (2008). Hippocampal amnesia impairs all manner of relational memory. Frontiers in Human Neuroscience, 2. doi: Artn 15 Doi 10.3389/Neuro.09.015.2008

Lavenex, P. B., Amaral, D. G., & Lavenex, P. (2006). Hippocampal lesion prevents spatial relational learning in adult macaque monkeys. Journal of Neuroscience, 26(17), 4546-4558. doi: Doi 10.1523/Jneurosci.5412-05.2006

Nemanic, S., Alvarado, M. C., & Bachevalier, J. (2004). The Hippocampal/parahippocampal regions and recognition memory: insights from visual paired comparison versus object-delayed nonmatching in monkeys. J Neurosci, 24(8), 2013-2026. doi: 10.1523/JNEUROSCI.3763-03.2004

Overman, W. H., Bachevalier, J., Sewell, F., & Drew, J. (1993). A comparison of children's performance on two recognition memory tasks: delayed nonmatch-to-sample versus visual paired-comparison. Dev Psychobiol, 26(6), 345-357. doi: 10.1002/dev.420260605

Pascalis, O., Hunkin, N. M., Bachevalier, J., & Mayes, A. R. (2009). Change in background context disrupts performance on visual paired comparison following hippocampal damage. Neuropsychologia, 47(10), 2107-2113. doi: DOI 10.1016/j.neuropsychologia.2009.04.001

Purves, D., Brannon, E.M., Cabeza, R., Huettel, S.A., LaBar, K.S., Platt, M.L., and Woldorff, M. (2008). Declarative Memory. *Principles of Cognitive Neuroscience.* 353-278. Sunderland, MA: Sinauer Associates.

Ross, R. S., & Slotnick, S. D. (2008). The hippocampus is preferentially associated with memory for spatial context. Journal of Cognitive Neuroscience, 20(3), 432-446.

Rudy, J. W. (2009). Context representations, context functions, and the parahippocampal-hippocampal system. Learn Mem, 16(10), 573-585. doi: 10.1101/lm.1494409

Ryan, J. D., Althoff, R. R., Whitlow, S., & Cohen, N. J. (2000). Amnesia is a deficit in relational memory. Psychological Science, 11(6), 454-461.

Scoville, W. B., & Milner, B. (1957). Loss of Recent Memory after Bilateral Hippocampal Lesions. Journal of Neurology Neurosurgery and Psychiatry, 20(1), 11-21.

Smith, C. N., Hopkins, R. O., & Squire, L. R. (2006). Experience-dependent eye movements, awareness, and hippocampus-dependent memory. Journal of Neuroscience, 26(44), 11304-11312. doi: Doi 10.1523/Jneurosci.3071-06.2006

Smith, C. N., & Squire, L. R. (2008). Experience-Dependent Eye Movements Reflect Hippocampus-Dependent (Aware) Memory. Journal of Neuroscience, 28(48), 12825-12833. doi: Doi 10.1523/Jneurosci.4542-08.2008

Smith, D. M., & Mizumori, S. J. Y. (2006). Hippocampal place cells, context, and episodic memory. Hippocampus, 16(9), 716-729. doi: Doi 10.1002/Hipo.20208

Smith, D. M., & Mizumori, S. J. Y. (2006). Learning-related development of context-specific neuronal responses to places and events The hippocampal role in context processing. Journal of Neuroscience, 26(12), 3154-3163. doi: Doi 0.1523/Jneurosci.3234-05.2006

Smith, S. M., Glenberg, A., & Bjork, R. A. (1978). Environmental Context and Human Memory. Memory & Cognition, 6(4), 342-353.

Squire, L. R., & Zolamorgan, S. (1991). The Medial Temporal-Lobe Memory System. Science, 253(5026), 1380-1386.

Zola, S. M., Squire, L. R., Teng, E., Stefanacci, L., Buffalo, E. A., & Clark, R. E. (2000). Impaired recognition memory in monkeys after damage limited to the hippocampal region. J Neurosci, 20(1), 451-463.

Table 1: Number of trials of each condition in each experiment used in the analyses. Trials in which the monkey did not fixate on the CR in the novel presentation were excluded, along with trials in which the animal showed little interest by viewing outside the screen. In experiment 3, LC denotes low context scenes and HC denotes high context scenes. Data from experiment 1 – TT is the same as experiment 2 - pre-lesion TT.

Experiment 1

	Animal				
	JN	**IW**	**MP**	**TT**	**Total**
Repeat without manipulation (Control)	133	120	139	87	**479**
Replaced Item Manipulation	130	127	137	86	**480**
Moved Item Manipulation	135	125	140	90	**490**
Total	398	372	416	263	**1449**

Experiment 2

	Animal	
	TT-Pre Lesion	**TT-Post Lesion**
Repeat without manipulation (Control)	87	78
Replaced Item Manipulation	86	70
Moved Item Manipulation	90	73
Total	**263**	**221**

Experiment 3

	Control Animals									
	JN		**IW**		**MP**		**Total**		**TT- Lesioned**	
	LC	**HC**	**LC**	**HC**	**LC**	**HC**	**LC**	**HC**	**LC**	**HC**
Repeat without manipulation (Control)	33	32	30	33	37	34	**100**	**99**	34	28
Replaced Item Manipulation	34	31	31	29	34	36	**99**	**96**	30	36
Moved Item Manipulation	32	36	32	34	33	30	**97**	**100**	36	34
Total	99	99	93	96	104	100	**296**	**295**	**100**	**98**

Figure 1: Outline of the Scene Manipulation Task (SCM). Eye movements were recorded with an infrared eye-tracking system. Monkeys were required to accumulate 10 seconds of looking time in each stimulus presentation. The white boxes outline the critical region (CR), which was not present during behavioral testing. Monkeys were rewarded between trials.

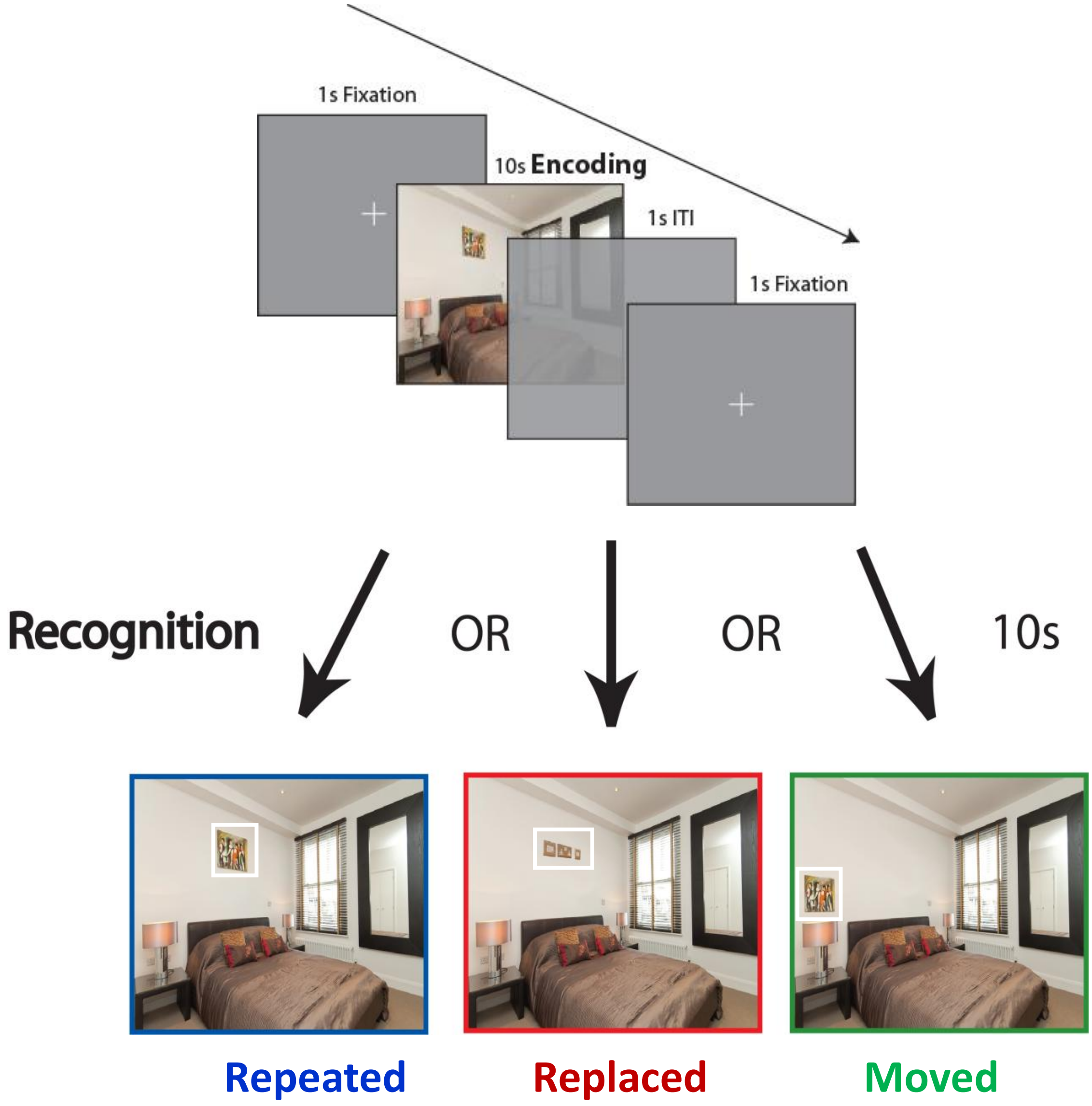


Figure 2: Example Scan Paths. Example scan paths when the scene was novel (A, Yellow); repeated without manipulation (B, Blue), repeated with one object replaced (C, Red), and repeated with one object moved (D, Green). Each monkey was presented with only one manipulation per scene; accordingly, these results are from three different monkeys.

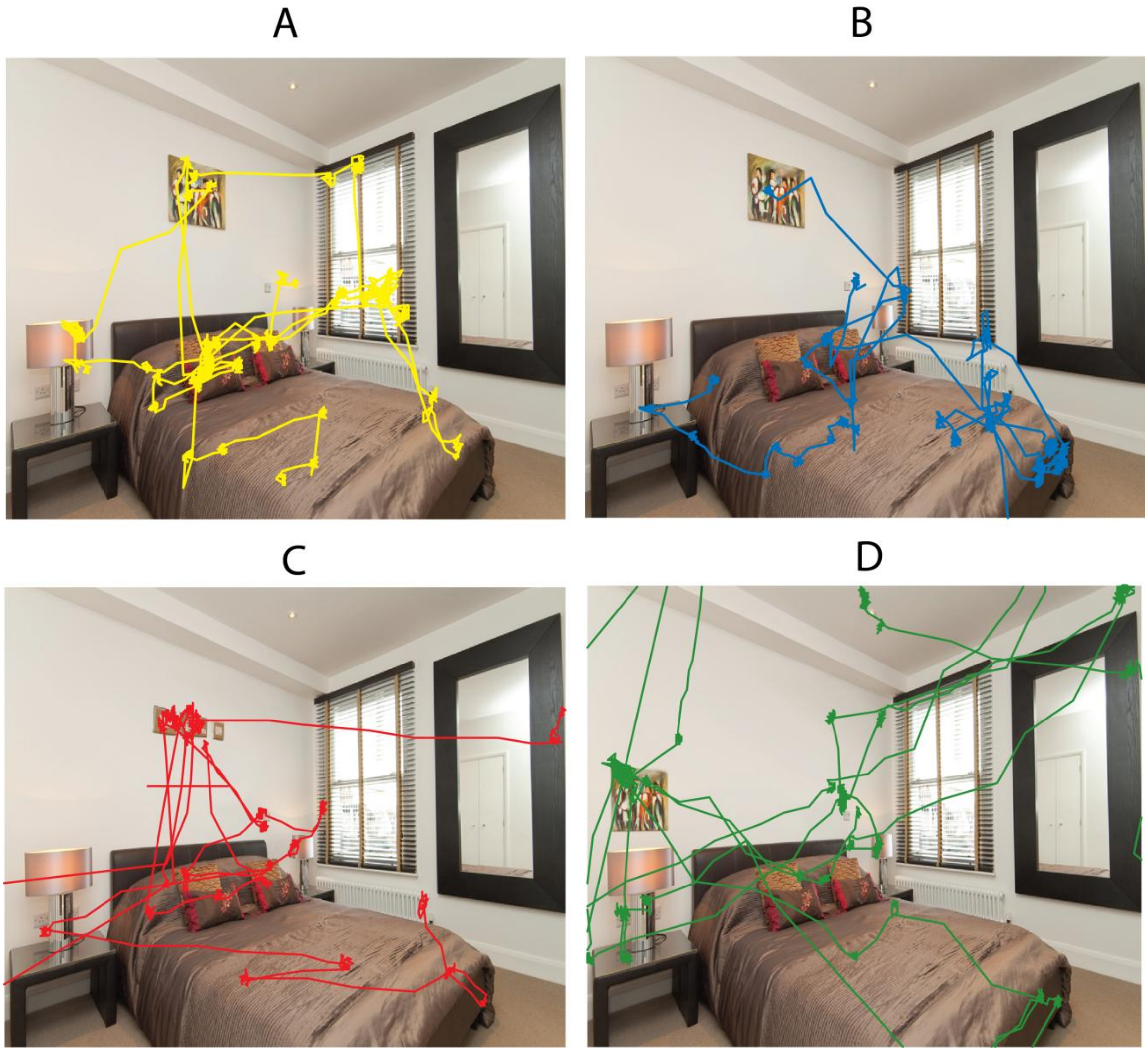


Figure 3: **Control monkeys demonstrate relational memory.** *Indicates significant differences (all p values <.01). N=44 sessions over 4 monkeys. These data were averaged across the full 10 seconds.

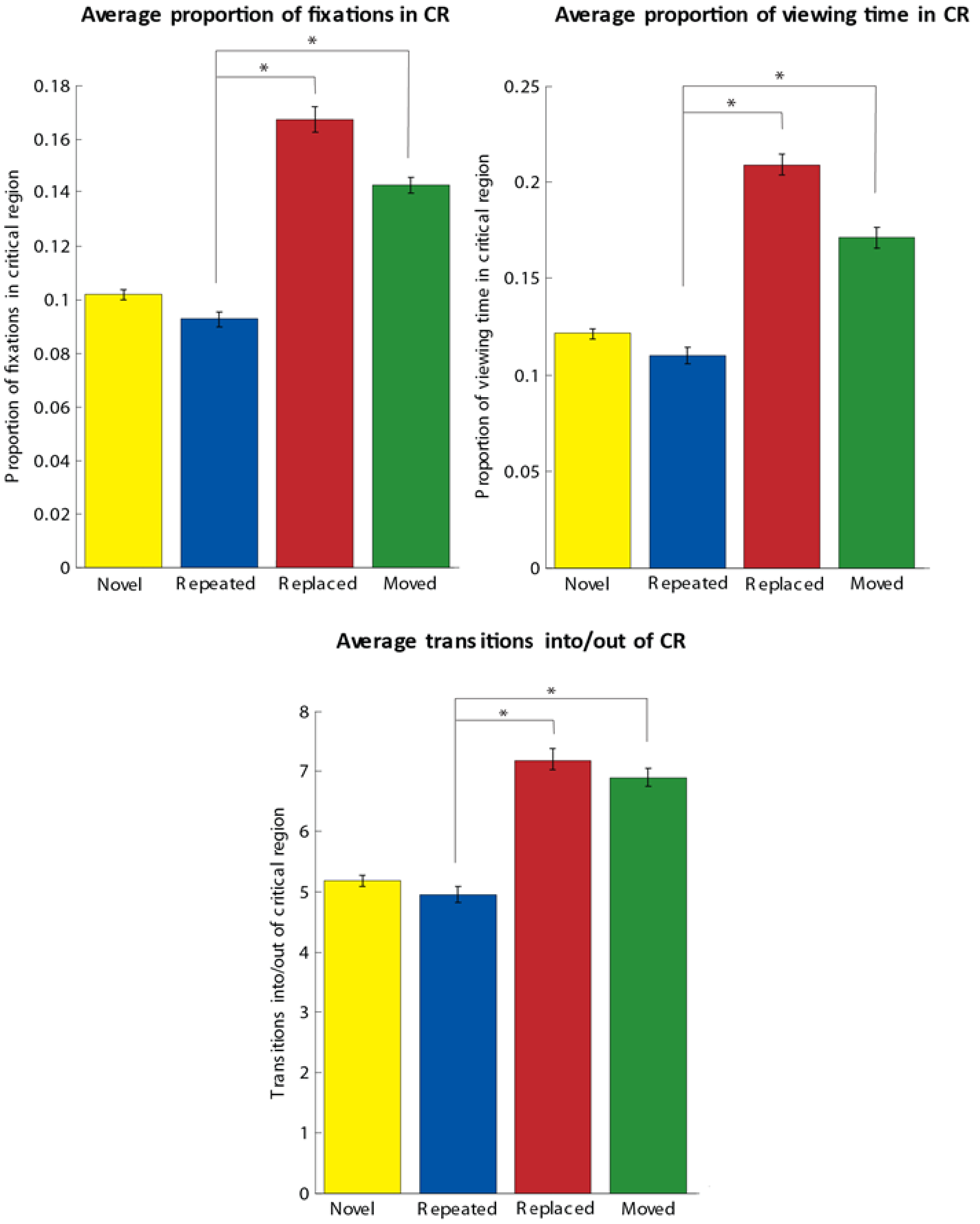


Figure 4: Time spent in the critical region (CR) over the course of the trial for control monkeys. Grey shaded area indicates significant difference between the second presentation without manipulation and with a replaced object and with a moved object (p<.01).

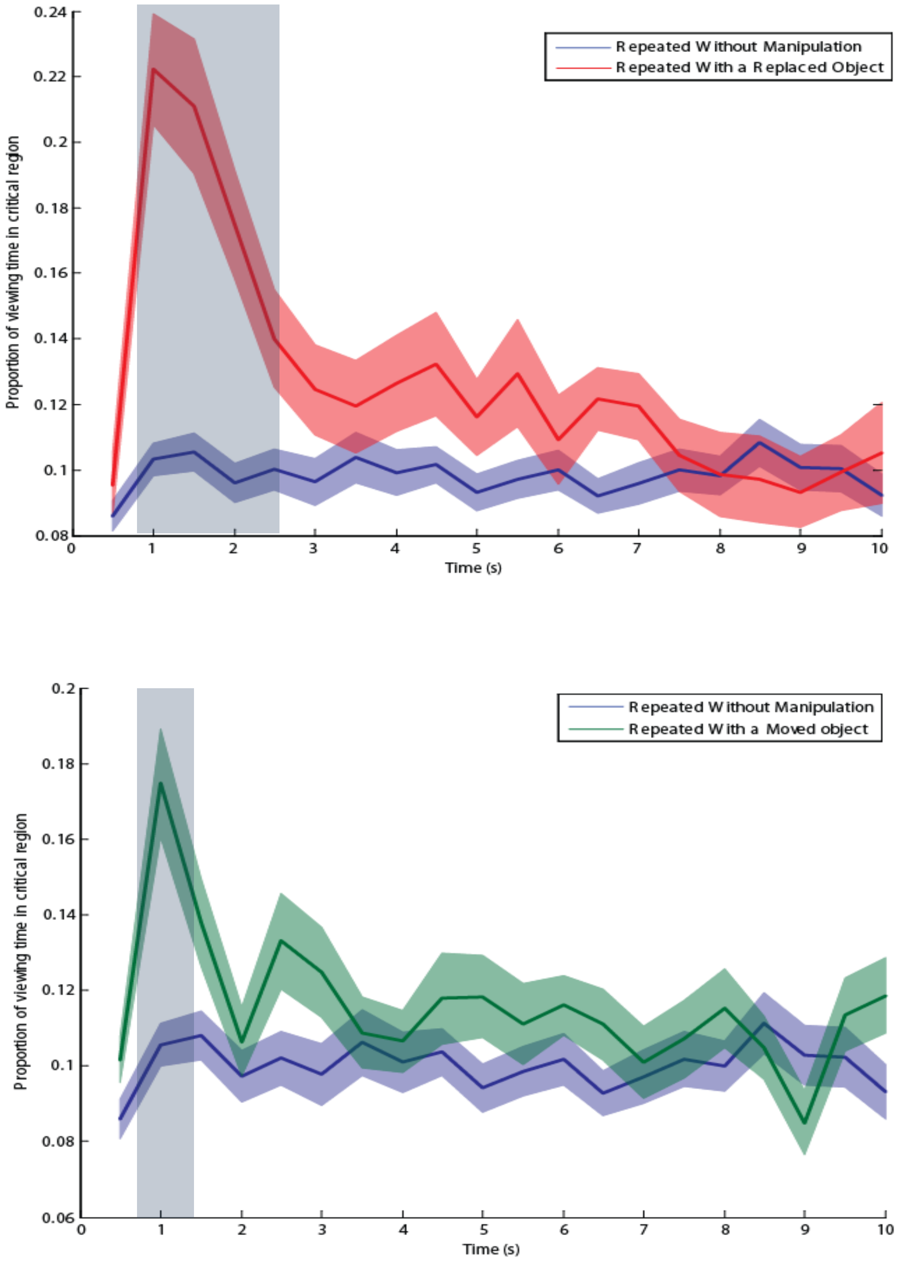


Figure 5: Control monkeys have a quick appreciation of manipulations. *Indicates significant increase in percentage of fixations and viewing time in, and transitions into/out of the critical region for the manipulated, compared to repeated but unmanipulated, scenes (p<.001). This data is averaged across the first 4s post stimulus onset.

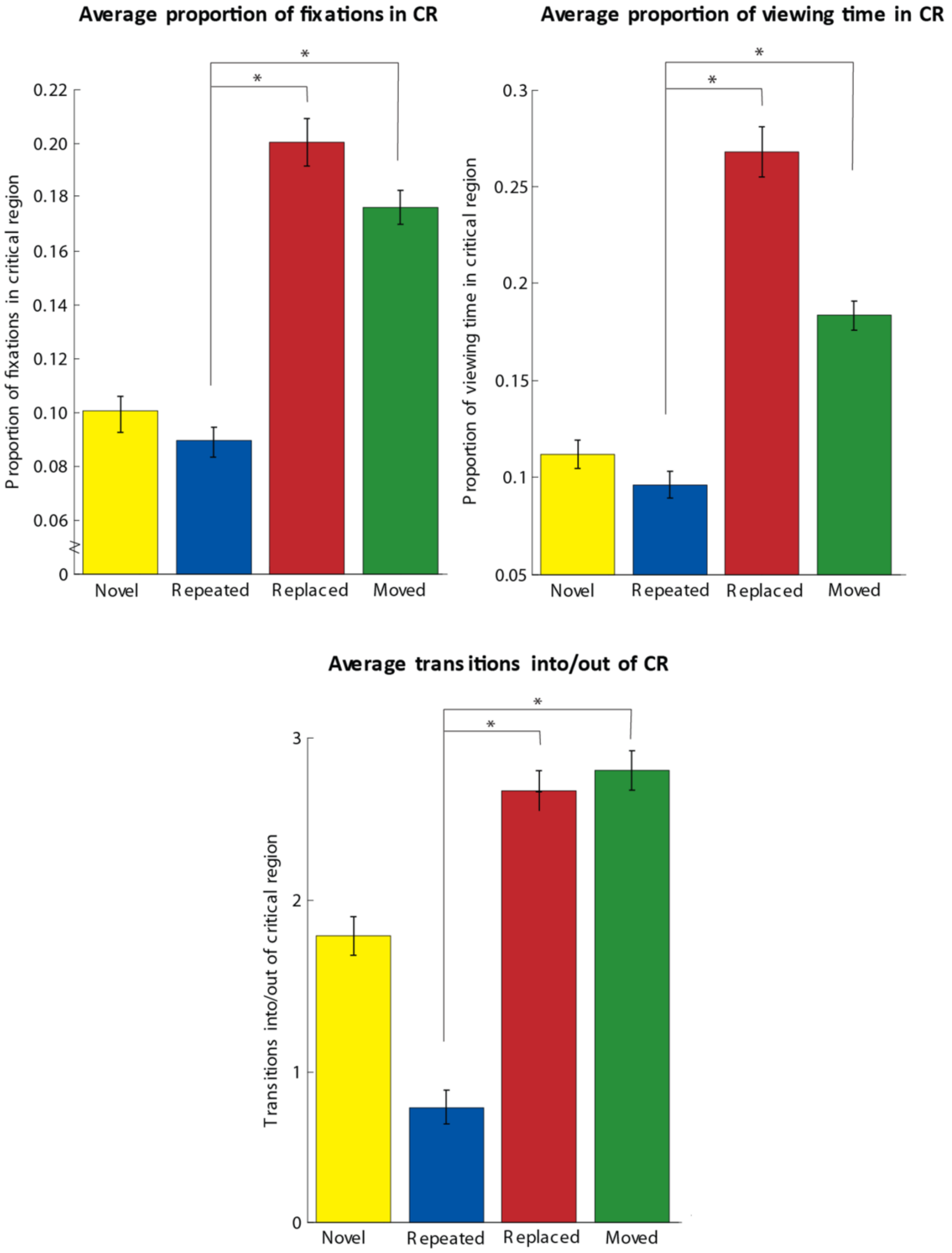


Figure 6: Hippocampal lesion FLAIR and T1. This shows the FLAIR and T1 MRI scans after bilateral ibotenic acid lesion of the hippocampus in one monkey. Post-operative MRI (FLAIR) revealed bilateral hyperintensity in the hippocampus, consistent with edema that arises from cell death caused by the neurotoxin injection. IA indicates distance in mm anterior to the inter-aural line.

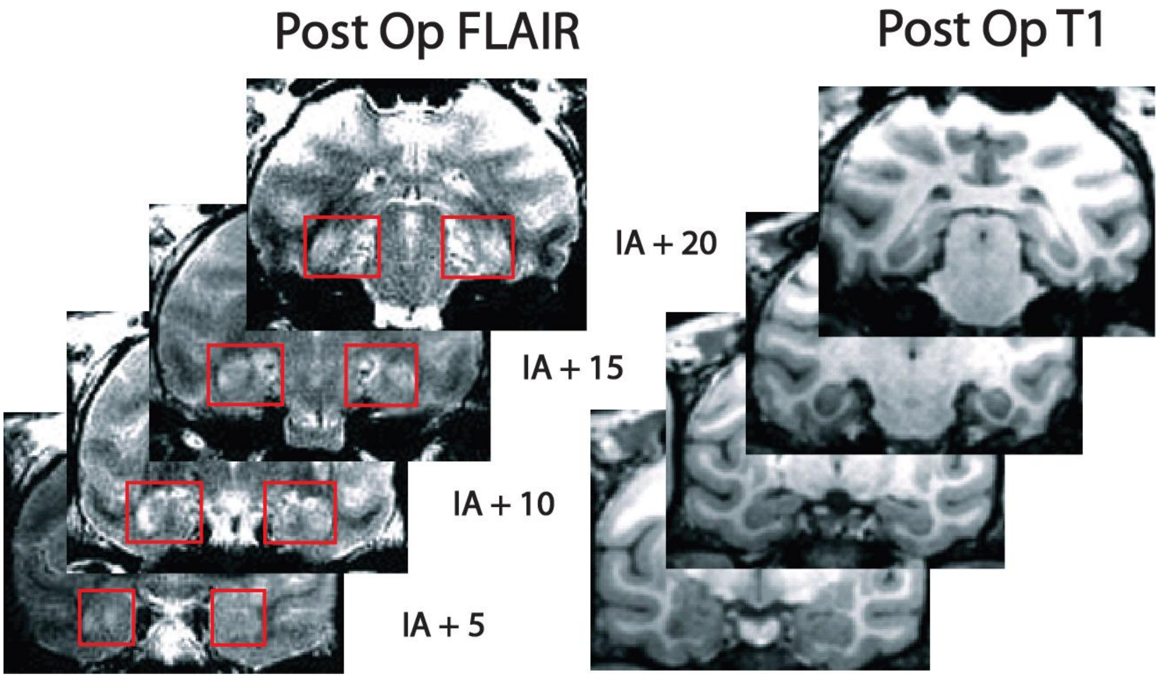


Figure 7: Relational memory impaired following hippocampal lesion. After the hippocampal lesion, the monkey failed to show a gaze preference for scenes with a move manipulation. 2-Way ANOVAs revealed significant interactions between the manipulations and the lesion (all p values <.01). For post-hoc t-tests, NS indicates no significant difference (all p values >.10). This data is averaged across the first 4s post stimulus onset. * Indicates a significant difference (p<.01)

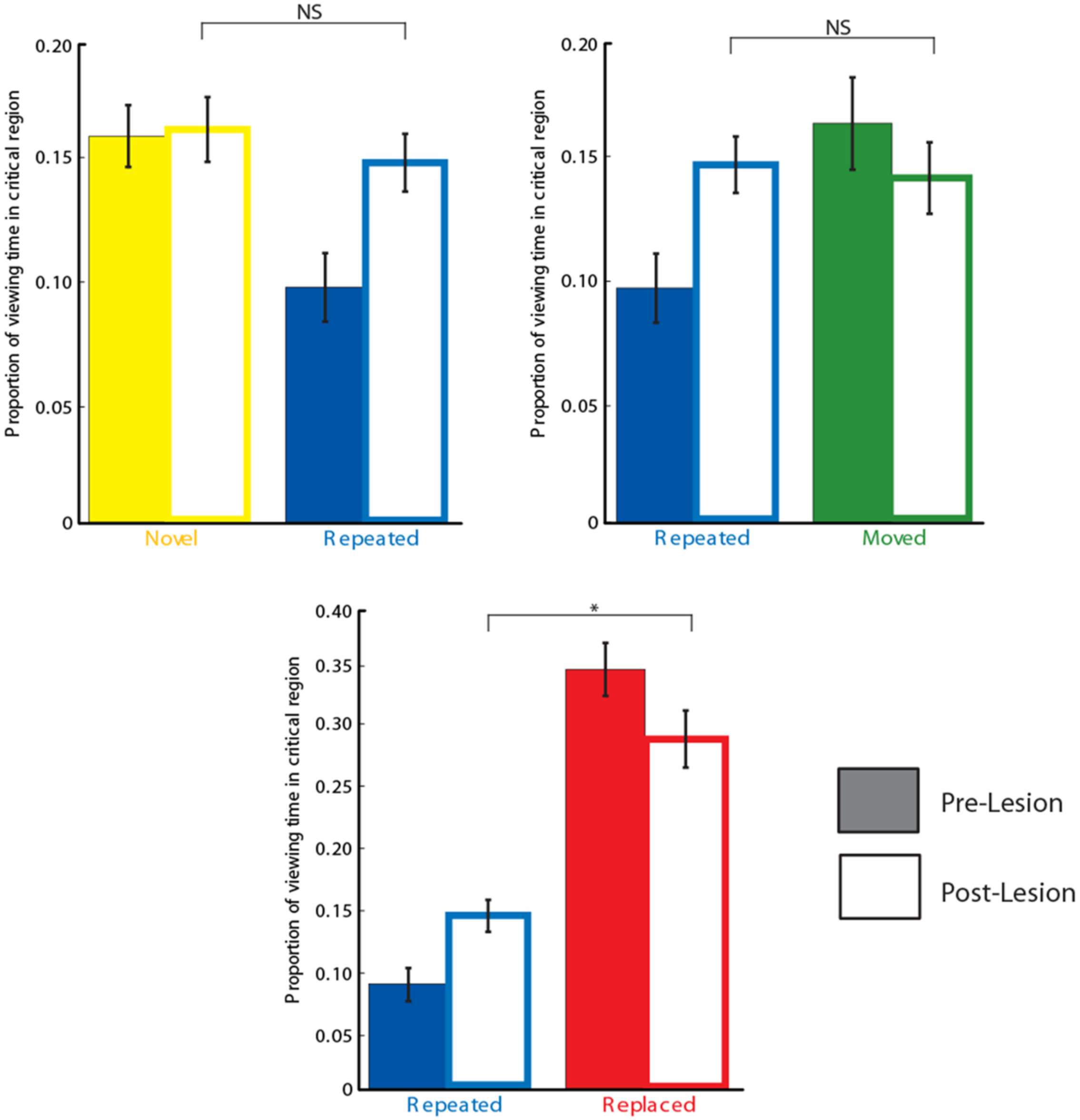


Figure 8: Relational memory depends on the hippocampus. In a subtraction analysis between relevant conditions, t-tests show significant differences in preferential looking within the CR for pre lesion and post lesion (all p values <.01). This data is averaged across the first 4s post stimulus onset.

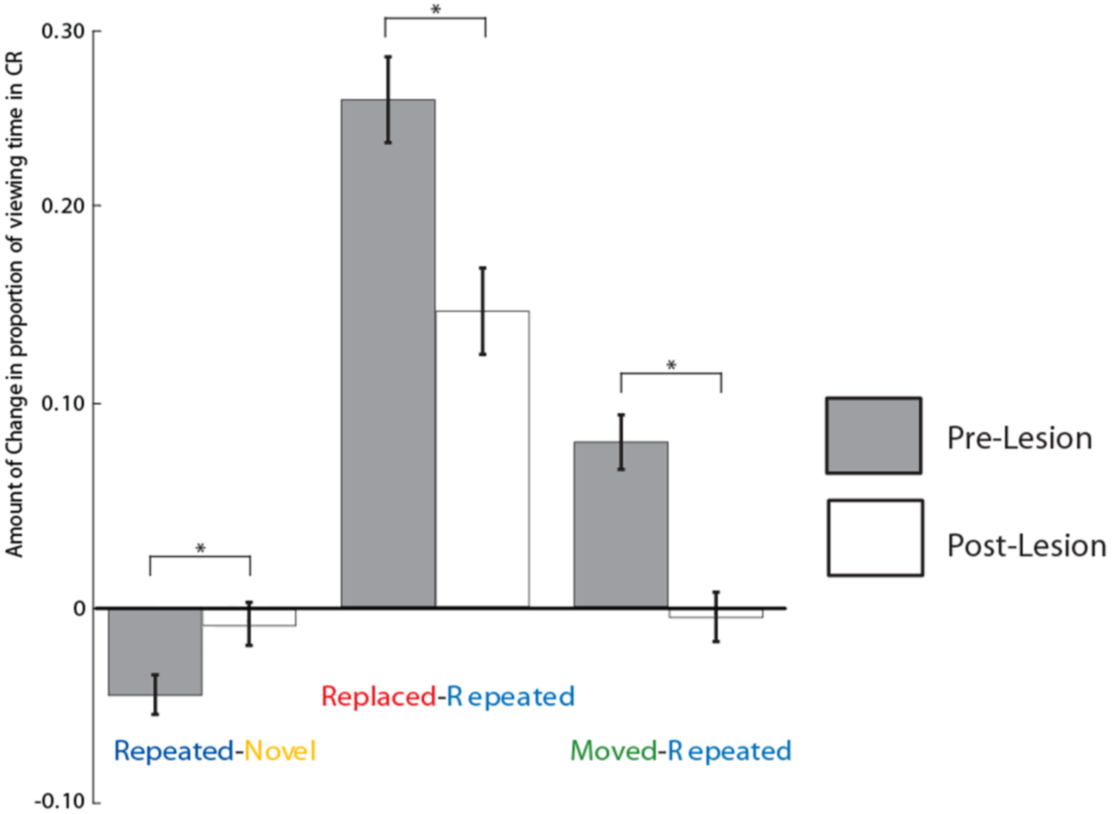


Figure 9: Time spent in the critical region (CR) over the course of the trial for the hippocampal-lesioned monkey. There was no significant difference between the second presentation without manipulation and with a replaced object or moved objects within the first 2 seconds. Grey shaded area indicates significant difference between the second presentation without manipulation and with a replaced object (p<.01).

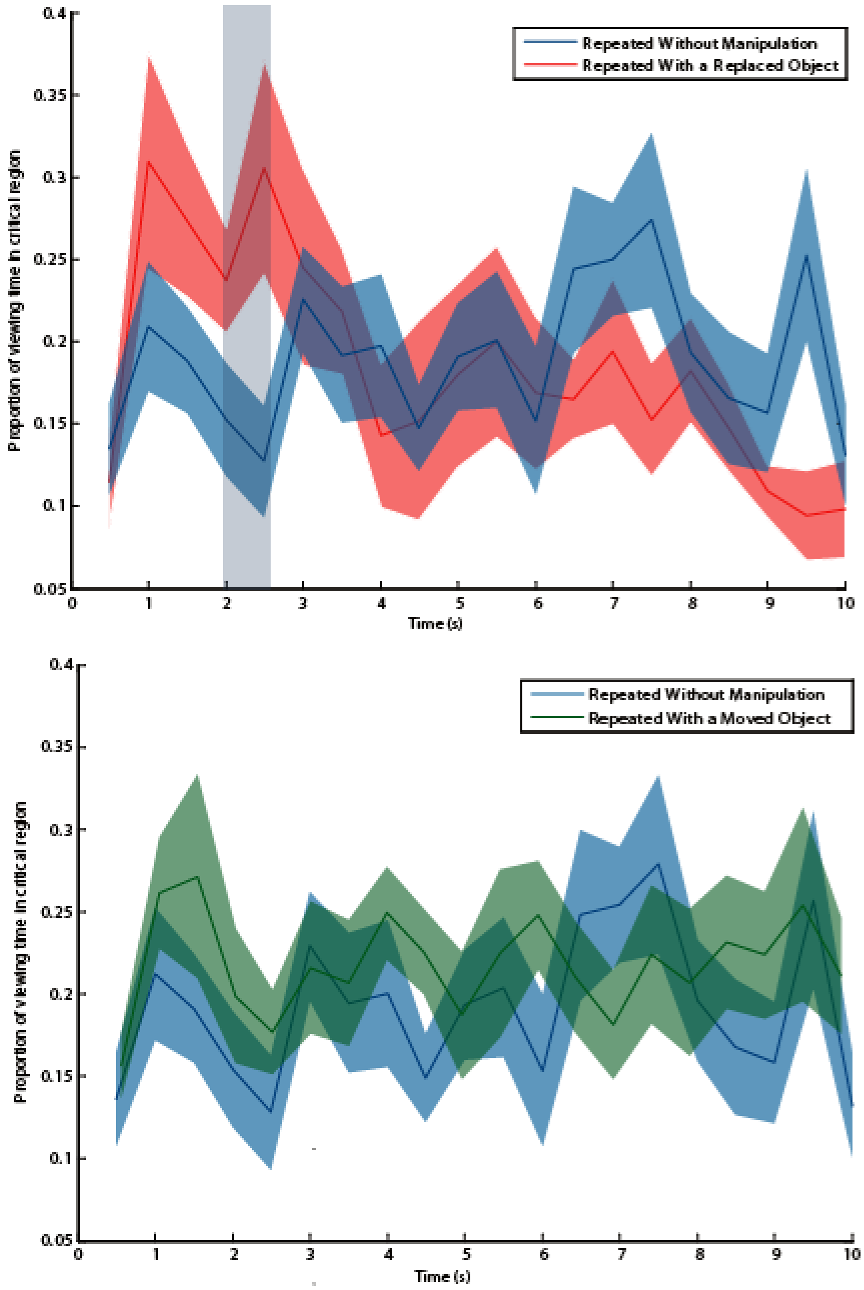


Figure 10: Examples of high context scenes and low context scenes used for experiment 3. Items were cropped out of a complex scene with around ten objects and were placed against a graded, single color background. These “salient” objects were chosen based on contrast in colors and defined borders.

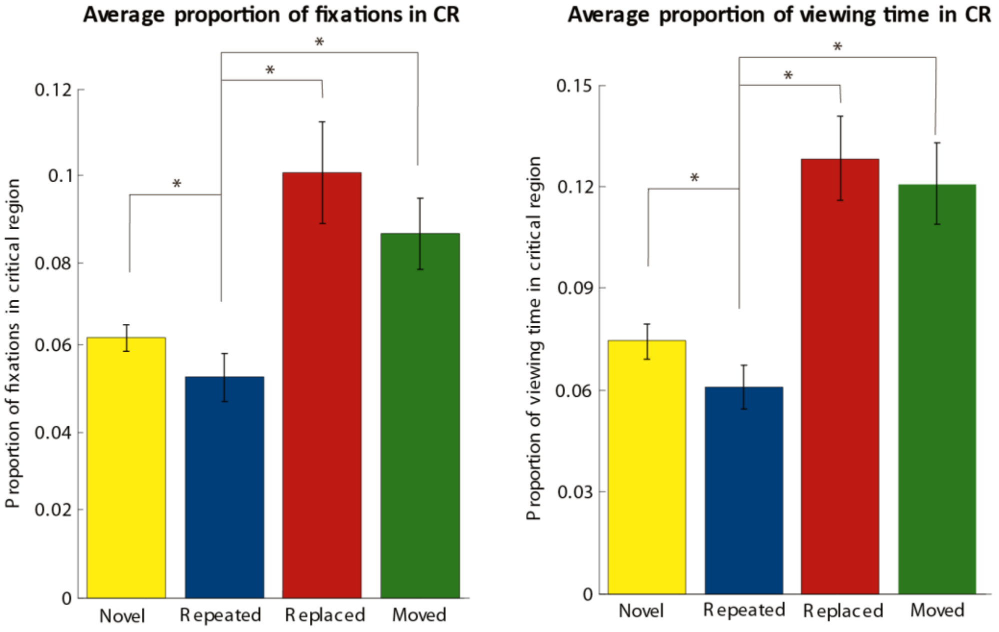
Low Context

High Context

Figure 11: Example scan patterns of scene with a low and high context shown to a normal monkey on two separate days, separated by at least one week. This demonstrates a similar viewing pattern and fixation pattern, indicating fixations were made to the cropped items even in the high context scenes.

Figure 12: Normal monkeys successfully form relational memories with high context and low context scenes. *Indicates significant differences in percentage of fixations and viewing time in the critical region (p<.05).

High Context



Low Context

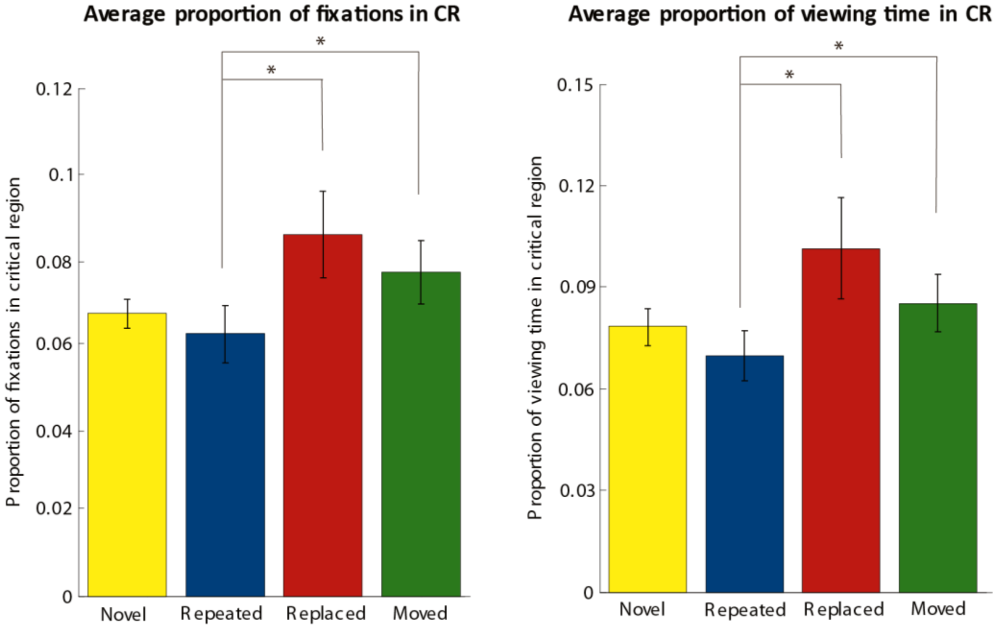


Figure 13: Normal monkeys are more successful in forming memories of scenes with a high context than those with low context. In a subtraction analysis between relevant conditions, t-tests show significant differences in preferential looking time within the CR for high and low context scenes between the manipulations and the control trials (all p values <.01).

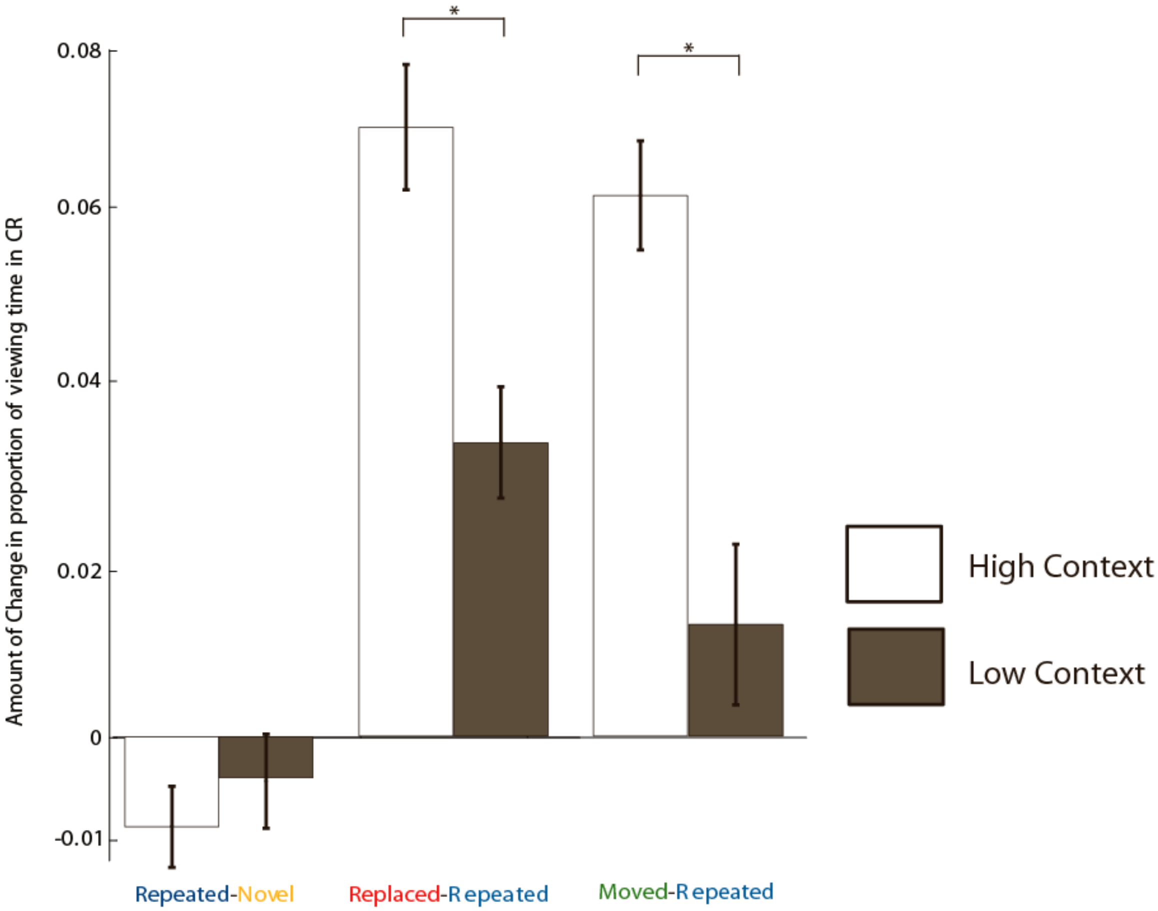


Figure 14: Hippocampus is necessary for successful relational memory formation only in representations with a high context, but is not necessary for those with a low context. The hippocampal lesioned monkey displayed normal viewing preferences in scenes with a low context. *Indicates significant differences in percentage of fixations and viewing time in the critical region (p<.05).

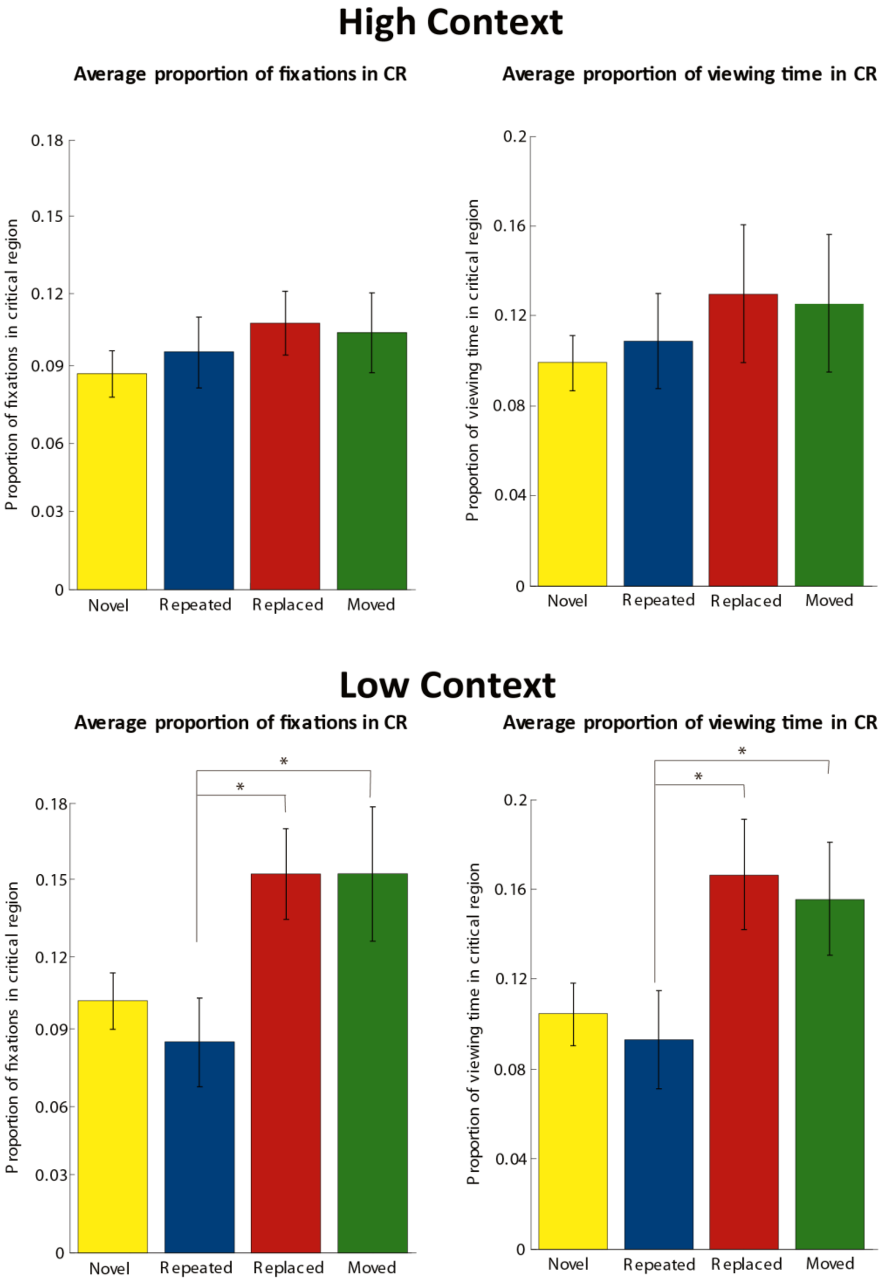


Figure 15: **The hippocampal-lesioned monkey displayed quick appreciation of the manipulated object in a low context scene.** This shows the time spent in the critical region (CR) over the course of the trial. Grey shaded area indicates significant difference between the second presentation without manipulation and with a replaced object and with a moved object (p<.01).