ANXIETY AND MEMORY:

THE INFLUENCE OF OXYTOCIN AND CORTICOSTERONE ON MEMORY RECONSOLIDATION IN RATS

By

RIANNE JANAI MICHAEL

A Thesis Submitted to The Honors College

In Partial Fulfillment of the Bachelors degree
With Honors in

Psychology

THE UNIVERSITY OF ARIZONA

M A Y 2 0 1 4

Approved by:

Dr. Jean-Marc Fellous
Department of Psychology

The University of Arizona Electronic Theses and Dissertations
Reproduction and Distribution Rights Form

The UA Campus Repository supports the dissemination and preservation of scholarship produced by University of Arizona faculty, researchers, and students. The University Library, in collaboration with the Honors College, has established a collection in the UA Campus Repository to share, archive, and preserve undergraduate Honors theses.

Theses that are submitted to the UA Campus Repository are available for public view. Submission of your thesis to the Repository provides an opportunity for you to showcase your work to graduate schools and future employers. It also allows for your work to be accessed by others in your discipline, enabling you to contribute to the knowledge base in your field. Your signature on this consent form will determine whether your thesis is included in the repository.

Name (Last, First, Middle)

Michael, Rianne, Janai

Degree title (eg BA, BS, BSE, BSB, BFA):

BS

Honors area (eg Molecular and Cellular Biology, English, Studio Art):

Psychology

Date thesis submitted to Honors College:

May 14, 2014

Title of Honors thesis:

Anxiety and Memory: The Influence of Oxytocin and Corticosterone on Memory Reconsolidation in Rats

The University of Arizona Library Release Agreement

I hereby grant to the University of Arizona Library the nonexclusive worldwide right to reproduce and distribute my dissertation or thesis and abstract (herein, the "licensed materials"), in whole or in part, in any and all media of distribution and in any format in existence now or developed in the future. I represent and warrant to the University of Arizona that the licensed materials are my original work, that I am the sole owner of all rights in and to the licensed materials, and that none of the licensed materials infringe or violate the rights of others. I further represent that I have obtained all necessary rights to permit the University of Arizona Library to reproduce and distribute any nonpublic third party software necessary to access, display, run or print my dissertation or thesis. I acknowledge that University of Arizona Library may elect not to distribute my dissertation or thesis in digital format if, in its reasonable judgment, it believes all such rights have not been secured.

☑ Yes, make my thesis available in the UA Campus Repository!

Student signature: Rianne Michael Date: 5/14/14

Thesis advisor signature: _____ Date: 5/14/2014

☐ No, do not release my thesis to the UA Campus Repository.

Student signature: _____ Date: _____

Last updated: 04/01/13

ABSTRACT

Psychologists have long been studying the structures and processes that are involved in storing and retrieving information which underlie the basis of human memory. A recent theory of memory processing, termed “reconsolidation,” has begun to complement the previously dominant consolidation theory of memory. Memory reconsolidation has been shown to rely heavily on spatial contexts, but the extent to which other types of cues can serve as a context for reconsolidation has not fully been investigated. In this study, the role of emotions on memory reconsolidation was examined by assessing the ability of distinct affective states induced through the acute administration of different doses of oxytocin (OXT) and corticosterone (CORT) to interfere with the reactivation of a previously consolidated memory in rats. It was found that the physiological effects produced through the administration of OXT and CORT may not have been sufficient to act as an emotional context as hypothesized; however, OXT was observed to enhance both memory consolidation and reconsolidation, while CORT was observed to enhance memory consolidation, but inhibit reconsolidation. This study was the first to examine the influence of emotionally-related drugs on memory reconsolidation and the results obtained suggest that this topic warrants further investigation.

Anxiety and Memory: The Influence of Oxytocin and Corticosterone on Memory Reconsolidation in Rats

The ability to construct memories has remained a critical aspect in the daily lives of various species as it plays an essential role in cognition as well as social and adaptive functioning. Indeed, conceptualization of our own personal identities relies on episodic memories of our past experiences and semantic memories of knowledge about our lives, such as our name and age. Therefore, numerous philosophers and psychologists throughout history have classified the various elements of memory as important topics for investigation. As a result, much is known about the process of memory formation today and many different factors have been identified that are able to influence it, including emotion, odors, and previous knowledge.

The role of emotions on memory and the mechanisms in which they exert their influence continue to be investigated. Theorists have debated over the ability of emotion to enhance or disrupt memories, with strong emotion being referred to as one of the six "lesser forms of aids to the memory" by Francis Bacon, but emotional persistence as one of the seven "sins of memory" by Daniel Schacter (LaBar and Cabeza, 2006). More recent research is showing support for both ideas, demonstrating diverse effects depending on the valence and arousal of the affective state of an emotion; however, studies of patients with lesions to the amygdala, a brain region proposed to mediate the influence of emotions on memory, have identified arousal, rather than valence, to be the more relevant factor in producing memory effects (LaBar and Cabeza, 2006). The stage of memory formation in which the emotion takes place is also a relevant factor, as different emotions have been shown to produce distinct effects during memory encoding, consolidation, and retrieval. For example, the occurrence of stress, an emotional state characterized by affect that is negative in valence and moderately high in arousal, during encoding and consolidation has been shown to improve memory performance, but when stress is experienced during retrieval, memory performance was found to be impaired (LaBar and Cabeza, 2006). Furthermore, particular categories of memory can be affected by emotion in different ways. For instance, negative affect has been shown to produce differential effects on associative and item memory (Bisby and Burgess, 2014). In this study, participants learned image-context associations that contained either a neutral or

negative image and were then asked to perform a ‘surprise’ memory test 24 hours later. Participants displayed better recall for the negative items than neutral items, but recall of the associated context of each item reversed this trend, with higher recall of the associated context for the neutral items. Thus, the study of emotional effects on memory has been shown to be a complex area of research with many factors to consider. Although many aspects relevant to determining the ways in which emotions influence memory have been identified and investigated, a majority of these studies have focused on negative emotions, as they are more easily induced as well as measured, and have neglected less distinguished types of memory processes, such as reconsolidation, leaving further areas for examination.

The use of animal models to study the influence of emotion on memory has proven to be a valuable tool as it permits precise control of experimental conditions and the use of pharmacological manipulations to allow specific types and stages of memory processing to be investigated as a function of specific emotional states. Since emotions cannot be explicitly demonstrated in animals, emotions are investigated through their behavioral response, which can be elicited by the administration of certain drugs. Two drugs that have recently attracted the attention of animal researchers, oxytocin (OXT) and corticosterone (CORT), have demonstrated effects that support their ability to be used for the induction of an affective state.

OXT is an endogenous neuropeptide that is released within distinct brain regions and into the circulatory system through the neurohypophysial terminal in response to stressful or social stimuli. The release of OXT has been shown to modulate a wide variety of behaviors, including maternal care and aggression, pair bonding, sexual behavior, social memory and support, as well as anxiety-related behavior and stress coping (Neumann, 2008). These features have lead OXT to be proposed as a promising target for psychotherapeutic intervention and treatment of numerous psychiatric illnesses, such as, anxiety disorders, social phobia, autism, and postpartum depression. The capability of OXT to represent an affective state in animals is demonstrated in its anxiolytic characteristics. When synthetic OXT was administered peripherally before an open-field test, rats exhibited significant reductions in anxiety-like behavior, but at higher doses, tended to display some sedative effects as well (Ayers et al., 2011). This observed

decrease in anxiety has been supported through measurements of physiological effects with OXT administration. Both female and male rats exhibit decreases in blood pressure, increases in nociceptive thresholds, and decreased levels of CORT when treated with (Petersson et al., 1999). Therefore, OXT administration in animals is able to generate effects that simulate a state of reduced stress and anxiety. These features are able to mimic the experience of an emotion distinguished by an affective state low in arousal and with positive valence. Despite these promising features, studies of OXT have focused on establishing its endogenous effects on distinct brain regions and behavior, rather than its more indirect applications to study emotion.

CORT is also able to produce effects that can represent an affective state, but on the opposite end of the spectrum as OXT. CORT is a steroid hormone, or more specifically, a glucocorticoid, that is released from the adrenal gland into the circulatory system as the final step in a cascade of neuroendocrine events associated with stress in response to novel or threatening stimuli. Once in the blood stream, glucocorticoids, such as CORT, act on target tissues throughout the body to confer physiological changes that enable organisms to manage acute stressors and eventually return them to a normal level of functioning (Gregus et al., 2005). Glucocorticoids have been shown to produce striking affective and psychotic disturbances in humans and can display elevated levels in certain psychopathological states, most notably depression, so their behavioral actions in animals has found particular clinical interest. CORT has been demonstrated to produce changes in the retention of passive avoidance behavior and open field activity, as well as an increase in escape behavior (Stone et al., 1988). Since CORT is partly responsible for the features of stress that produce its affective response, administration of CORT can be used to simulate the experience of stress, thus providing the ability to elicit this emotion in animals. With the affective nature of stress being characterized by high arousal and negative valence, CORT functions as an appropriate counterpart for the investigation of emotion on memory with OXT, which produces mostly the opposite effects. As mentioned previously, many of the effects of stress on memory have already been examined and were indicated to have differing effects on the various stages of memory formation. However, not all aspects of memory have been investigated.

Although the consolidation theory of memory suggests that once a memory is stored, it remains stable over time, recent research has identified a new aspect of memory formation that allows memories to be updated, sometimes resulting in significant alterations (Tronson and Taylor, 2007). This new stage in the process of memory formation has been termed “reconsolidation” (Nadel et al., 2012). In reconsolidation, reactivation of a memory through remembering renders it labile for a period of time, causing initiation of a restabilization process in which updating of the memory can occur. This process has been modeled in a positively motivated task in rats (Jones et al., 2012).

Impairment of as well as addition to a memory have both been demonstrated as consequences of reconsolidation. In one study, memory of a finger-tapping sequence was impaired in participants by its rehearsal prior to learning a different sequence (Walker et al., 2003). Reactivation was shown to cause this effect since no impairment was seen when the original sequence was not rehearsed. In addition, recollection of the procedure used to learn a prior list of objects before learning a new list of objects caused participants to remember objects from both lists when prompted only to recall the original list of objects (Hupbach et al., 2007). This shows that reactivation allowed the memory of the original list of objects to be updated with information from the second list of objects during reconsolidation, which is supported by the lack of intrusions seen when participants were not asked to recall the previous procedure; however, subsequent research has indicated that the spatial context was more effective to cue the reactivation than being asked to recall the previous procedure (Hupbach et al., 2008).

Animal models have shown useful in exposing the effects of different pharmacological agents on reconsolidation and have given insight to specific brain regions involved in these processes (Lee and Everitt, 2008; Miller and Marshall, 2005; Wang et al., 2005; Nader and Einarsson, 2010). Similar to human studies, recent animal research has examined addition of new information into consolidated memories through reconsolidation, but only using stressful water maze tasks (Morris et al., 2006). Jones et al. (2012) was the first to examine reconsolidation using an appetitively motivated paradigm to eliminate any effects of stress. In this study, rats were subjected to list learning tasks modeled after those used in the Hupbach et al. (2007) study on humans. Different spatial contexts were utilized to reactivate the memory of the previously learned

list and were able to yield comparative results to those found by Hupbach et al. (2007). Although the effects of reconsolidation have been demonstrated in a wide range of species and memory paradigms, the effect of emotions on reconsolidation has yet to be investigated.

To date, spatial context has been demonstrated to be the most relevant factor in promoting reconsolidation in both humans and rats, but the extent to which different cues can serve as a context for reconsolidation warrants further investigation. The various factors that comprise a spatial context, such as the visual, tactile, and olfactory components, provides a strong collection of cues that can serve to reactivate a memory, so other contexts that provide various cues for recollection may serve to promote reconsolidation as well. One such context could be that which is created by the experience of an emotion, which features diverse physiological, cognitive, and behavioral variations that can serve as cues. The current study attempts to expand on previous research by examining the ability of specific induced emotional states via pharmacological manipulations to serve as a context which prompts memory reconsolidation in rats.

Rats were presented with list learning tasks through a procedure adapted from Jones et al. (2012) Experiment 1. In order to replicate the Reminder context from this study, which provided cues to allow reactivation of a previous memory, rats received a subcutaneous injection of a control substance, either saline or sesame oil, to imitate the stable contextual cues used across each list learning task and during recall. Two No Reminder contexts, which discouraged memory reactivation, were also simulated through the use of a subcutaneous injection of either OXT or CORT on Day 2 that is meant to be similar to the variation in spatial contextual cues through a change in affective state. The results of the Reminder condition will provide insight to the base level of reconsolidation, indicated by number of intrusions, which is expected for a stable context. With this information, the effect of variations in affective state can be more readily assessed. It is hypothesized that the stability of the Reminder context due to lack of spatial or affective variations will yield high rates of intrusions during recall because the rats will not have a cue to differentiate between lists, but that the affective states induced by OXT and CORT in the No Reminder context will be able to serve as a cue to allow differentiation among

the learned lists and thus cause less intrusions to occur, similar to the results indicated in the Reminder and No Reminder groups from Jones et al. (2012) Experiment 1.

Method

In order to reproduce the results found in Jones et al. (2012) Experiment 1, methods were constructed in accordance with those originally described. The following sections outline the most relevant aspects of the study design and any modifications that were applied.

Animals

Fifteen adult (7-12 month-old) male Brown Norway rats were used across the various experimental conditions. Rats were housed in separate Plexiglas home cages maintained on a reversed 24 hour light/dim cycle. Due to the nocturnal nature of rats, experiments were conducted during the dim phase of the cycle. Rats were also food deprived to 80-85% of their *ad libitum* weight to encourage participation in the appetitively motivated list learning tasks. Although most rats were reused, the number of experiments performed and conditions that were tested varied among individual rats, resulting in assorted numbers of animals across each condition. The number of rats tested in each condition is reported in the figures as well as in the results; however, an individual rat may have been used more than once in a specific condition. In order to ensure that the rats did not develop a preference for lists of feeders from previous experiments, at least 1 day of drug-free random training was performed between experiments, during which all eight feeders were repeatedly cued in random order. All procedures took place in accordance with the animal care guidelines of the University of Arizona.

Stimuli and Measures

Rats were tested in an open-field circular arena that was 5 feet in diameter and lined with a 1 foot tall wall around the periphery that contained eight evenly spaced feeders. Each feeder was equipped with a LED light to signal the list of feeders to be learned. Onset of the LED signal could be delayed to assess whether the rats were visiting the list of feeders by memory alone. Feeders used for each list dispensed a sugar water reward (0.12 g/mL, small drop) when triggered by the approach of a rat. An overhead camera was used to track the rats as they performed each task. Feeders and lights were automatically controlled by a computer with custom-written software. Affective states characterized by either a diminished or heightened level of anxiety were induced in rats with subcutaneous injections (0.5 mL) of OXT (0.1, 1.0, 1.25, or 1.5 mg/kg) and CORT

(10, 20, or 30 mg/kg), respectively. To achieve the desired dosages, OXT and CORT were dissolved in sterile saline (0.9%) and sesame oil, respectively. Control groups received injections of either saline or sesame oil.

Study Design and Procedures

All rats were pretrained to visit feeders with a blinking light to earn a sugar water reward. After pretraining, rats went through the experimental design depicted in Figure 1. During the active phase on Day 1 of the experiment, rats were cued by blinking lights to visit three predetermined feeders presented in random order (e.g. List 1 = 3, 5, 7) while in their natural affective state (Fig. 1A). Once they visited each feeder 50 times (150 rewards), the light cues were delayed by 15 seconds to encourage the rats to use their memory when choosing which feeders to visit. If rats did not visit the correct feeder within 15 sec, the light cue was activated for that feeder. In order to demonstrate that a list had been learned, rats needed to accomplish two criteria: 1) 15 correct list feeders were visited consecutively, 2) no more than two feeders were cued (i.e., rats beat the light at least 13 out of 15 times). During the active phase on Day 2 of the experiment, the task performed was identical to Day 1, except rats were cued to learn a different list of feeders (e.g. List 2 = 1, 4, 6). On Day 2, pharmacological manipulations were performed before the start of the initial sleep period, affording drugs adequate time to become assimilated, and constituted the different experimental conditions (Fig. 1B). Once assimilated, the

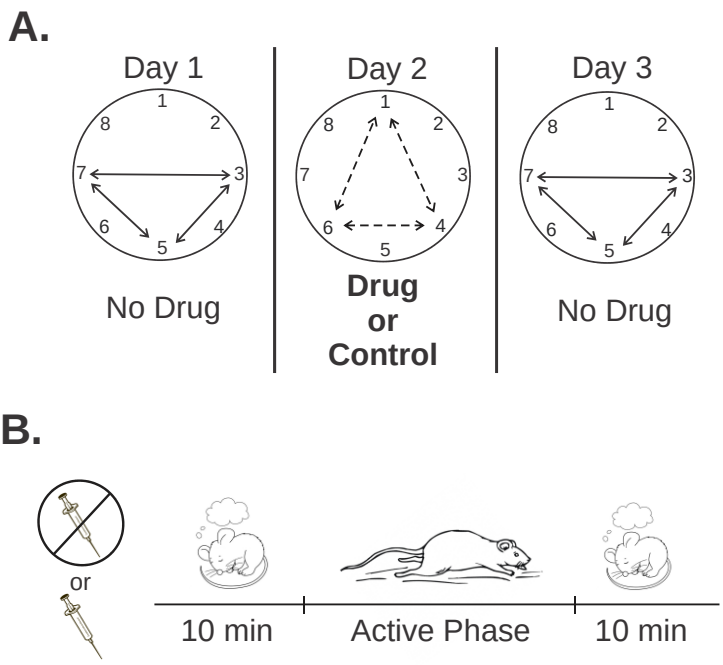

Figure 1. Experimental design. (A) Overarching experimental paradigm. Rats learned List 1 on Day 1 without the influence of any drug. Rats Learned List 2 on Day 2 while under the influence of a drug or control solution. Rats were cued to recall List 1 on Day 3 without the influence of any drug. (B) Daily experimental task. Rats would either receive no injection or an injection of a drug or control substance. A 10 minute sleep period occurred before and after the active phase of the task.

drugs are meant to be active throughout the entire task. Peripheral administration of synthetic OXT has recently been shown to produce significantly elevated plasma levels of OXT for up to two hours after the initial injection in rats (Neumann et al., 2013). Elevated levels compared to baseline were also apparent in the amygdala and hippocampus for at least one hour after administration, although significant changes were not demonstrated in these regions. In addition, peripheral administration of CORT displayed heightened levels in serum as well as the hypothalamus, pituitary gland, and nucleus tractus solitaries two hours after its application in control rats (Nguyen et al., 2000). Importantly, neither of these studies demonstrated effects of OXT or CORT that lasted 24 hours after they were administered. Therefore, each drug can be presumed to be active throughout the task, without affecting subsequent tasks. The various Control and Drug conditions used in this experiment were designed to correspond to the Reminder and No Reminder conditions in Jones et al (2012), respectively. To resemble the Reminder condition, rats were injected with an inert substance, either saline or sesame oil, to allow learning of List 2 to occur in a similar affective state as List 1. To resemble the No Reminder condition, rats were injected with an anxiolytic or anxiogenic drug, either OXT or CORT, respectively, to allow learning of List 2 to occur in a different affective state as List 1. During the active phase on Day 3, rats were cued to recall List 1 while in their natural affective state (drug-free, the same affective state as on Day 1). At the beginning of the task, a light cue was immediately displayed for a feeder from List 1 to prompt rats for its recall, then all subsequent light cues were delayed by 15 seconds. Only feeders belonging to List 1 were able to be cued and dispense rewards. The task continued until rats achieved the same criteria employed to establish list learning.

Data Collection and Analysis

The feeder choices of each rat were recorded by the both the experimenter and computer program. Visits to each feeder type (List 1, List 2, and No List) are expressed as a percentage of the total number of feeders visited and have been normalized to account for the different amounts of each feeder type using Microsoft Excel. To calculate the percent correct recall and intrusions for each rat, the proportion of No List feeders was subtracted from the proportions of List 1 and List 2 feeders, respectively, in order to control for baseline errors. Visits to feeders that were cued by lights during recall were

not included in analysis. To calculate list learning errors for each rat, the number of incorrect feeders visited (List 2 and No List for Day 1, List 1 and No List for Day 2) were added.

Time points for the start of the task, onset of cue delay, and end of task were recorded in the computer program with experimenter intervention. To calculate the time taken to prompt the cue delay and to learn each list for each rat, the time point for start of task was subtracted from the time point for onset of cue delay and the time point for onset of cue delay was subtracted from the time point for end of task, respectively.

Differences between groups were analyzed using either unpaired two-tailed t-tests or Mann-Whitney Rank Sum Tests. Data analysis with a Mann-Whitney Rank Sum Test was only performed if a t-test was not achievable due to a failed test of normality or equal variance. Data was excluded from analysis if calculations resulted in 0% recall or intrusions or values that were greater than two standard deviations from the mean.

Results

Drug Effects on Learning and Behavior

The use of pharmacological manipulations to induce affective states characterized by either a diminished or heightened level of anxiety may have the ability to cause secondary effects on learning and behavior when applied to the body peripherally. In order to determine whether the acute administrations of OXT and CORT used in this experiment produced any supplementary effects that would bias the results obtained when analyzing their ability to influence memory reconsolidation, several measures were taken to assess learning and behavior under the influence of each drug: time to prompt cue delay, list learning time, and list learning errors (discussed below). Since these measures aim to determine the influence of each drug, only data from Day 2, when the injection of each drug took place, is evaluated.

Establishment of control measurements.

Multiple conditions from throughout the experiment were able to constitute potential control groups for comparison in this analysis (Fig. 2). First, and most apparent, would be the comparison between the drug and its respective solute, which allows the individualistic effect of the drug to be assessed. Since OXT and CORT are differential in their solubility, a different substance, saline or sesame oil, respectively, must be used for each comparison. In identifying an appropriate solute for each drug, it is critical that the chosen solute does not have a meaningful effect on the variables being examined. Therefore, neither saline nor sesame oil should have any significant effects on learning or behavior and should thus be comparable in their results. For this reason, collapsing the saline and sesame oil groups to obtain a larger group that represents the effects that receiving an injection may have on learning and behavior can serve as a second control condition, possibly with more statistical value. Finally, comparison of the effects of each drug to learning and behavior while in a natural state can be examined by observing differences between the results obtained on Day 1. Regardless of the experimental condition, all rats performed the list learning task on Day 1 without receiving an injection or being under the influence of any drug. By collapsing all of the results gathered on Day 1 into a single group for comparison, a control group that represents normal learning and behavior can be achieved.

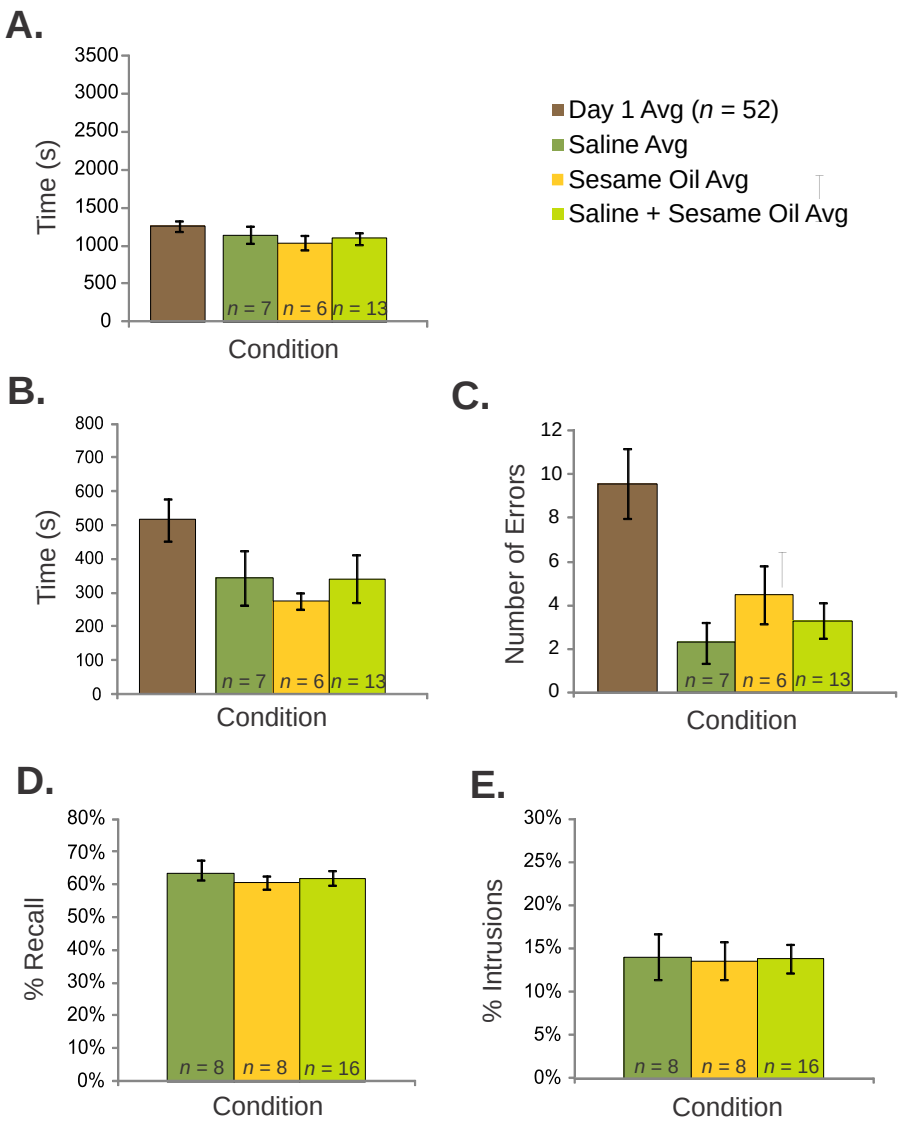

Figure 2. Comparison among controls. Error bars represent standard errors of means. (A) Mean time taken to visit 150 cued feeders during active phase on Day 1 and Day 2 while learning List 1 and List 2, respectively. (B) Mean time taken to reach criterion during active phase after cue has been delayed on Day 1 and Day 2. (C) Mean number of feeders visited belonging to List 2 or No List and List 1 or No List after cue has been delayed on Day 1 and Day 2, respectively. (D) Mean percentage of feeders visited belonging to List 1 during recall of List 1 on Day 3. (E) Mean percentage of feeders visited belonging to List 2 during recall of List 1 on Day 3.

In order to assess the ability of each of the proposed control groups to serve as an adequate control, it is important to identify whether there are any significant differences between these groups that need to be accounted for. A comparison of each proposed control group among the various measures analyzed is depicted in Figure 2. No statistical

differences were found among the controls for any of the learning (Fig. 2B, C) or behavioral (Fig. 2A) assessments.

The amount of time taken, in seconds, for rats to visit 150 cued feeders, thus signaling the start of the cue delay (Fig. 2A), in the saline (M = 1144.09, SD = 297.03, n = 7) and sesame oil (M = 1042.58, SD = 229.93, n = 6) groups did not differ significantly (t-test, p = .511). Combination of the data from these groups (M = 1118.21, SD = 249.54, n = 13) did not alter values in such a way that produced significant changes from either the saline (t-test, p = .720) or sesame oil (t-test, p = .668) group when assessed individually. Comparison to values obtained on Day 1 (M = 1259.29, SD = 449.00, n = 52) showed no differences among the saline (Mann-Whitney Rank Sum Test, p = 665), sesame oil (Mann-Whitney Rank Sum Test, p = .180), or combined saline and sesame oil (Mann-Whitney Rank Sum Test, p = .241) groups.

The amount of time taken, in seconds, for rats to complete the learning criteria once the cue has been delayed (Fig. 2B) did not differ significantly (Mann-Whitney Rank Sum Test, p = .836) between the saline (M = 344.12, SD = 210.56, n = 7) and sesame oil (M = 276.17, SD = 56.37, n = 6) groups. When combined (M = 342.49, SD = 251.11, n = 13), these groups showed no significant differences from their individual saline (t-test, p = .710) and sesame oil (Mann-Whitney Rank Sum Test, p = .859) counterparts. Comparison to the Day 1 values (M = 516.27, SD = 448.45, n = 52) revealed no significant variation from those obtained in the saline (Mann-Whitney Rank Sum Test, p = .550), sesame oil (Mann-Whitney Rank Sum Test, p = .301), or combined saline and sesame oil (Mann-Whitney Rank Sum Test, p = .275) groups.

The number of incorrect list feeders visited by rats when demonstrating list learning after the cue has been delayed (Fig. 2C) was not significantly different (t-test, p = .189) when comparing the saline (M = 2.29, SD = 2.43, n = 7) and sesame oil (M = 4.50, SD = 3.27, n = 6) groups. No further differences were detected between the individual saline (Mann-Whitney Rank Sum Test, p = .416) and sesame oil (t-test, p = .439) groups upon comparison to the values of these groups combined (M = 3.67, SD = 3.11, n = 13). Day 1 results (M = 9.72, SD = 11.63, n = 52) did not differ significantly from the sesame oil (Mann-Whitney Rank Sum Test, p = .710) or combined saline and sesame oil group (Mann-Whitney Rank Sum Test, p = .146), although the individual saline group did

demonstrate a marginally significant decrease in the amount of incorrect feeders attended (Mann-Whitney Rank Sum Test, $p = .085$).

Throughout the comparisons between the proposed control groups for the learning and behavioral assessments, no significant discrepancies were identified among the saline, sesame oil, combined saline and sesame oil, or Day 1 groups. These results indicate that the subtle differences between each condition did not drastically affect the learning or behavior of the rats and that each group is comparable in its ability to serve as a control. For instance, the finding that neither the saline nor sesame oil group displayed significant differences from each other justified the decision to collapse their data into a larger control group. Because no differences were found between the saline and sesame oil groups for any measure, they were thus found to be analogous in their effects, making their results comparable and worthy of combination. Also, no differences were observed between the saline and sesame groups and the Day 1 group, supporting the use of saline and sesame oil as a solute for their respective drug. In order to determine that the effects observed in the assessments of learning and behavior were attributable solely to the influence of each drug, any effects of saline or sesame oil on these measures needed to be characterized. In identifying an appropriate solute for each drug, it is desired that neither solute would have an effect on the measures to be analyzed to make the influence of each drug more perceivable. A lack of differences between these solutes and the results from Day 1 indicate that when administered alone, each solute produced results comparable to those obtained when rats are in their natural state, validating their neutral effect. In addition, the absence of differences between the Day 1 results and all other controls demonstrated that the administration of an injection before the task did not alter subsequent learning or behavior. Since no injection occurred on Day 1, differences among the other controls could represent either effects of the solute or effects of the injection. As no differences were observed, it can be concluded that neither the solute nor the injection influenced learning or behavior. Furthermore, the absence of differences between the Day 1 results and the other controls also illustrated that the pervious learning of List 1 had no effect on learning or behavior for List 2. It is possible that learning a separate list in the same context as a previous list was able to interfere with later learning abilities or alter behavior, but the correspondence between learning and behavior on Day

1 and Day 2, exposed by the various controls, refuted this idea. Therefore, in the following analyses of learning and behavior, comparison to the solute of the drug, to values of both drug solutes combined, and to values obtained on Day 1 will be used as controls to determine the influence of each drug.

Influence of oxytocin.

To determine the influence of OXT on learning and behavior, three separate measures were performed to analyze its effects. First, the time taken to prompt the cue delay was used to assess any behavioral effects (Fig. 3A), then list learning time and list learning errors were used to assess any effects on learning (Fig. 3B, C).

As mentioned previously, the first portion of the active phase on Day 1 and Day 2 consisted of 150 cued rewards to allow rats to become adequately acquainted with each list of feeders before the cue was delayed. Since the feeders corresponding to each list were cued during this portion of the task, posing no demands on memory or requirements of learning, any variation between conditions in the amount of time taken to visit the 150 cued feeders and prompt the cue delay can be attributed to changes in behavior and used to assess the behavioral effects of OXT at each of the administered doses. Similarly, measures from the second portion of the active phase on Day 1 and Day 2 can be used to assess the effect of each OXT dose on learning. Since the cues were delayed during this portion of the task, rats needed to rely on their memory to visit the correct feeders in order to complete the criteria and demonstrate that a list had been learned. Differences in rats' abilities to achieve the learning criteria can reflect how easily a list was able to be learned and used to establish any effects on learning. Both the amount of time taken and the number of incorrect feeders visited before the list learning criteria had been accomplished can serve as measures to gauge the extent of difficulty experienced during list learning and will be used for this analysis.

OXT was observed to produce significant effects on behavior at some of the doses administered (Fig. 3A). The 1.5 mg/kg OXT group (M = 1969.97, SD = 749.09, n = 3) exhibited the greatest difference from the controls, with the amount of time taken, in seconds, for rats to prompt the cue delay being significantly greater than the saline (t-test, p = .030), combined saline and sesame oil (Mann-Whitney Rank Sum Test, p = .015), and Day 1 (Mann-Whitney Rank Sum Test, p = .025) control groups. A significant increase

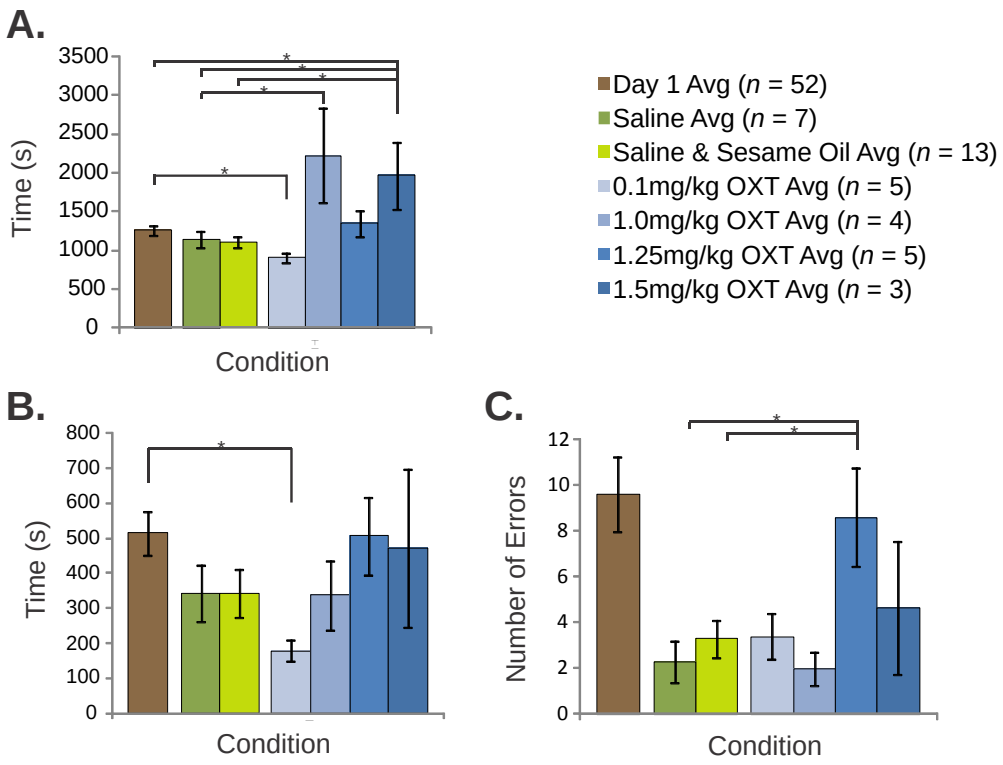

Figure 3. Effects of OXT on learning and behavior. Error bars represent standard errors of means. (A) Mean time taken to visit 150 cued feeders during active phase on Day 1 and Day 2 while learning List 1 and List 2, respectively. (B) Mean time taken to reach criterion during active phase after cue has been delayed on Day 1 and Day 2. (C) Mean number of feeders visited belonging to List 2 or No List and List 1 or No List after cue has been delayed on Day 1 and Day 2, respectively. (*) = P < .05

in time to prompt the cue delay was also observed in the 1.0 mg/kg OXT group (M = 2223.39, SD = 1217.70, n = 4) when compared to the saline control (t-test, p = .046), but this effect diminished to marginal significance when data from the saline and sesame oil groups were combined for comparison (Mann-Whitney Rank Sum Test, p = .062) and when comparing to Day 1 (Mann-Whitney Rank Sum Test, p = .095). The lowest OXT dose, 0.1 mg/kg, created the opposite effect, significantly decreasing time to prompt the cue delay (M = 912.06, SD = 135.26, n = 5), but this effect was only seen when weighed against Day 1 (Mann-Whitney Rank Sum Test, p = .025). No effects on behavior were demonstrated in the 1.25 mg/kg OXT group (M = 1354.27, SD = 371.09, n = 5).

Little influence of OXT on list learning time was found among the various doses administered (Fig. 3B). Only the 0.1 mg/kg OXT group (M = 179.80, SD = 64.60, n = 5) displayed a significant difference from any of the controls, showing a decrease in the

amount of time taken, in seconds, for rats to complete criterion compared to the Day 1 control (Mann-Whitney Rank Sum Test, p = .031). Although no difference was apparent in the saline control (t-test, p = .126), this effect was also observed with marginal significance when the saline and sesame oil data were combined (t-test, p = .090). Marginal significance (t-test, p = .061) was also identified through comparison to the combined saline and sesame oil control for the 1.25 mg/kg OXT group (M = 507.48, SD = 247.29, n = 5), but indicating effects in the reversed direction with an increase in time taken to complete criterion. Neither the 1.0 mg/kg (M = 338.36, SD = 197.98, n = 4) nor 1.5 mg/kg (M = 471.29, SD = 392.01, n = 3) OXT groups produced noticeable variations from any of the controls.

The number of list learning errors also showed little influence from the various OXT doses administered (Fig. 3C). A significant increase in the number of incorrect feeders visited before rats completed criterion was displayed in the 1.25 mg/kg OXT group (M = 8.60, SD = 4.83, n = 5) for both the saline (t-test, p = .013) and combined saline and sesame oil (t-test, p = .011) controls, but not the Day 1 control (Mann-Whitney Rank Sum Test, p = .411). No differences between any of the control groups and 0.1 mg/kg (M = 4.50, SD = 3.27, n = 6), 1.0 mg/kg (M = 4.50, SD = 3.27, n = 6), and 1.5 mg/kg (M = 4.50, SD = 3.27, n = 6) OXT groups were identified.

Across the measures analyzed to identify any relevant effects of peripherally administered OXT, no dose was found to consistently influence both learning and behavior. It was anticipated that the larger group size created from the combination of data from the saline and sesame oil groups as a control would be able to provide more statistical value and, indeed, a lower p-value was obtained upon comparison with this control relative to its individual components in these cases and most cases to follow. The largest OXT dose, 1.5 mg/kg, displayed consistent differences from each control, exhibiting an increase in time taken to prompt the cue delay. Therefore, OXT, when administered at high doses, was shown to slow down performance during the task, thus demonstrating some minor behavioral effects. These effects were not present during learning, as no significant differences were found in the list learning time or list learning errors. Some of the lower OXT doses displayed significant variation from controls in the behavioral and learning assessments, but none of the effects of these doses persisted

among all controls or across both learning measures. Consequently, the peripheral administration OXT does not appear to effect learning at any dose, but when administered at high doses, some behavioral effects are exhibited.

Influence of corticosterone.

The effects of CORT were analyzed in an equivalent manner as OXT in order to identify any influences on learning or behavior. First, the time taken to prompt the cue delay was used to assess any behavioral effects (Fig. 4A), then list learning time and list learning errors were used to assess any effects on learning (Fig. 4B, C).

A dose effect on behavior was observed among the CORT groups (Fig. 4A). This effect was characterized by lower doses displaying a greater amount of time taken, in seconds, for rats to prompt the cue delay, with a regression back towards baseline as the dose was increased. Accordingly, the 10 mg/kg CORT group (M = 2477.77, SD = 1332.97, n = 4) showed the greatest increase in time taken to prompt the cue delay, differing significantly from the sesame oil (Mann-Whitney Rank Sum Test, p = .019), combined saline and sesame oil (Mann-Whitney Rank Sum Test, p = .015), and Day 1 (Mann-Whitney Rank Sum Test, p = .016) controls. Increasing to the 20 mg/kg CORT dose (M = 1371.09, SD = 149.05, n = 6) continued to display significant differences from the sesame oil (t-test, p = .015) and combined saline and sesame oil controls (Mann-Whitney Rank Sum Test, p = .032), but differences from the Day 1 control were no longer evident (Mann-Whitney Rank Sum Test, p = .105). No significant differences from controls were apparent in the 30 mg/kg CORT group (M = 954.97, SD = 78.20, n = 6), but a marginally significant decrease in time to prompt the cue delay was found from the Day 1 control (Mann-Whitney Rank Sum Test, p = .061).

A slight influence of CORT on list learning time was found among the administered doses (Fig. 4B). Only the highest CORT dose, 30 mg/kg (M = 199.24, SD = 49.12, n = 6), produced significant differences from any of the controls, demonstrating a decrease in time taken, in seconds, to complete the list learning criteria from the sesame oil (t-test, p = .030) and Day 1 (Mann-Whitney Rank Sum Test, p = .033) controls. Combination of the saline and sesame oil data reduced this effect and only displayed marginal significance (Mann-Whitney Rank Sum Test, p = .087). Neither the 10 mg/kg (M = 400.46, SD =

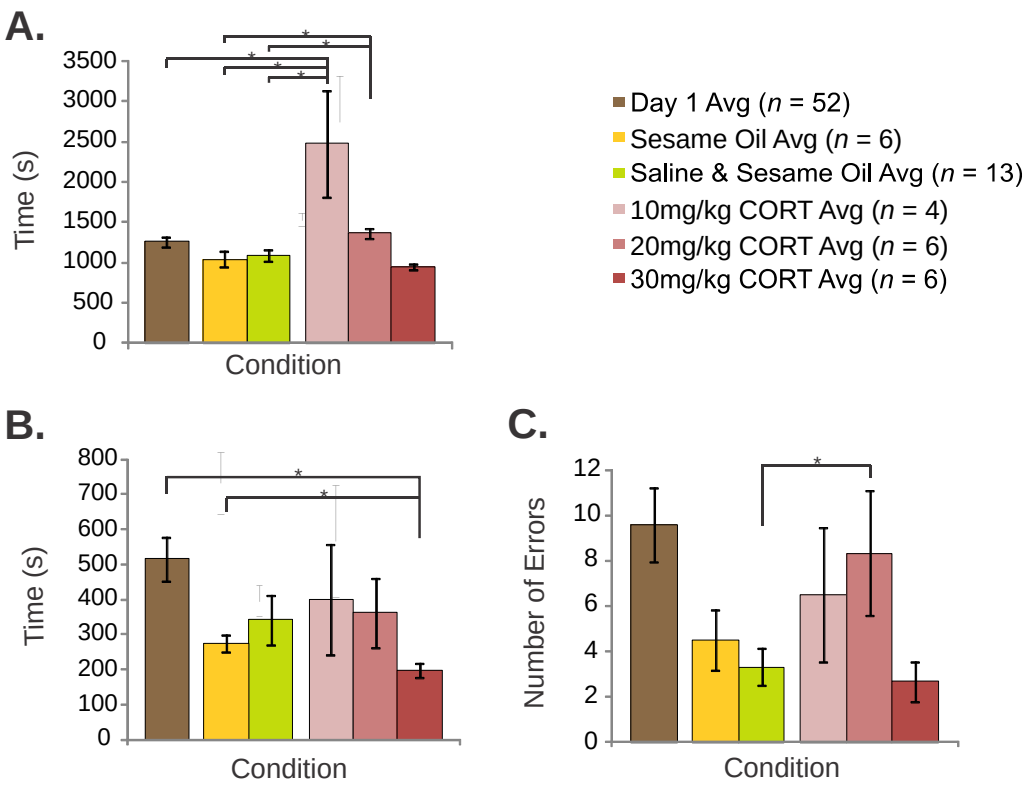

Figure 4. Effects of CORT on learning and behavior. Error bars represent standard errors of means. (A) Mean time taken to visit 150 cued feeders during active phase on Day 1 and Day 2 while learning List 1 and List 2, respectively. (B) Mean time taken to reach criterion during active phase after cue has been delayed on Day 1 and Day 2. (C) Mean number of feeders visited belonging to List 2 or No List and List 1 or No List after cue has been delayed on Day 1 and Day 2, respectively. (*) = P < .05

317.56, *n* = 4) nor 20 mg/kg (*M* = 363.60, *SD* = 240.21, *n* = 6) CORT groups revealed any significant differences from the controls.

CORT also displayed a slight influence on the number of list learning errors among the doses administered (Fig. 4C). The 20 mg/kg CORT group (*M* = 8.33, *SD* = 6.71, *n* = 6) was found to significantly increase the number of incorrect feeders visited by rats before completing criterion when compared to the combined saline and sesame oil control (t-test, *p* = .034), but this effect was not seen when examining sesame oil alone (t-test, *p* = .237). No other effects were displayed in either the 10 mg/kg (*M* = 6.50, *SD* = 5.92, *n* = 4) or 30 mg/kg (*M* = 2.67, *SD* = 2.16, *n* = 6) CORT groups for any of the controls.

The measures analyzed to identify any relevant effects elicited by the peripheral administration of CORT did not reveal a consistent influence on both learning and behavior for any dose. Similarly to the OXT analysis, only one group was found to produce

a consistent difference across all three controls. The effect observed was analogous to that seen in OXT, displaying an increase in time taken to prompt the cue delay in the behavioral assessment, but was found for the lowest dose, rather than highest. Therefore, CORT, when administered at a low dose, demonstrated some minor behavioral effects by slowing down performance during the task. Learning, however, was not influenced by these effects, as no significant differences were found in the list learning time or list learning errors. Although the other doses of CORT displayed some significant variations from the controls in the behavioral and learning assessments, none of the effects persisted among all controls or across both measures of learning. As a result, peripherally administrated CORT does not appear to effect learning at any dose, but can induce some effects on behavior when administered at a low dose.

Drug Effects on Reconsolidation

The main goal of this study was to identify whether the phenomena associated with distinct, pharmacologically induced affective states are able to influence reactivation of previously stored memories and, consequently, affect reconsolidation. Since the design of this study was closely modeled after Experiment 1 by Jones et al. (2012), the results obtained were analyzed a corresponding manner. Therefore, in order to characterize the effects of pharmacologically induced affective states on reconsolidation, the relative amounts of recall and intrusions (defined below) exhibited on Day 3 were examined for the various OXT and CORT groups.

Control conditions.

Since any general effects that learning List 2 might have had on the maintenance or retrieval of the List 1 memory were already discredited by Jones et al. (2012), establishment of controls for the following analyses focused on distinguishing the effects of each drug. The conditions previously employed as control groups to assess the influence of each drug on learning and behavior may also serve as relevant controls for comparison in this analysis. Differences between the tasks performed on Day 1 and Day 3 caused the Day 1 control group to be discarded, as a direct comparison to results could no longer be established. However, the comparison of each drug to its respective solute and to the data of both solutes combined still remained applicable for the reasons originally described and were investigated further.

In order to determine whether there are any significant differences between the remaining controls that need to be taken into consideration, the control groups were compared among the measures to be analyzed. No statistical differences were identified between any of the controls for either of the reconsolidation assessments (Fig. 2C, D).

The proportion of List 1 and List 2 feeders visited on Day 3 remained stable across all controls (Fig. 2C, D). The saline (M = 63.40, SD = 11.25, n = 8) and sesame oil (M = 60.69, SD = 5.85, n = 8) groups did not demonstrate significant differences in the percentages of List 1 recall (t-test, p = .555). No additional differences were revealed across the saline (t-test, p = .748) or sesame oil (t-test, p = .698) groups when compared to the data of these groups combined (M = 62.04, SD = 8.77, n = 16). Likewise, no significant variation between the saline (M = 14.03, SD = 7.48, n = 8) and sesame oil (M = 13.59, SD = 6.17, n = 8) groups was identified for the percentage of List 2 intrusions (t-test, p = .900). Results from the combination of the saline and sesame oil data (M = 13.81, SD = 6.63, n = 16) did not differ from the individual data of the saline (t-test, p = .942) or sesame oil (Mann-Whitney Rank Sum Test, p = .976) groups.

The findings relative to the saline, sesame oil, and combined saline and sesame oil controls that were demonstrated in the assessments of reconsolidation resembled those previously found in the learning and behavioral assessments and supported the same conclusions that were reached.

Influence of oxytocin.

To determine whether OXT is able to influence reconsolidation, two related measures were used to analyze its effects. Percent recall and percent intrusions were both evaluated to assess any impact on reconsolidation (Fig. 5A, B).

By allowing previously stored memories to become integrated with new information, reconsolidation is able to alter the stability of memories over time. Thus, measures that are able to demonstrate memory stability or provide evidence that a memory has been integrated with new information can serve as effective tools to evaluate reconsolidation. The proportions of List 1 and List 2 feeders visited by rats on Day 3 allowed reconsolidation to be assessed along both of these lines in each condition. For instance, since rats were cued to recall List 1 on Day 3, the level of accuracy in which List 1 was able to be recalled, demonstrated by the proportion of List 1 feeders visited, can

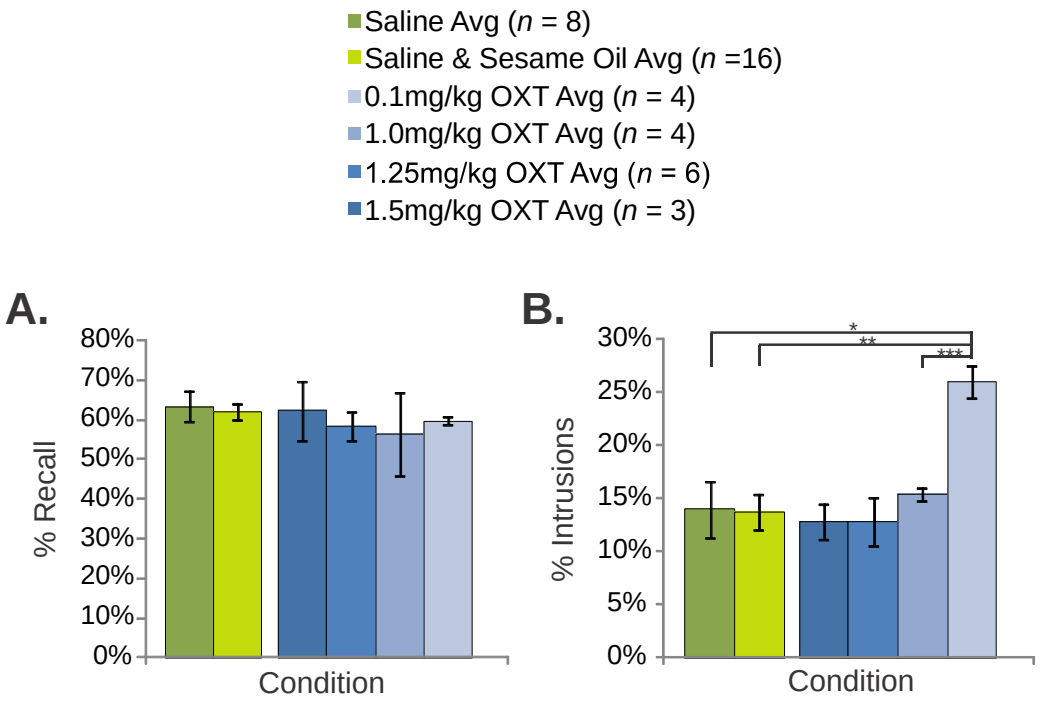

Figure 5. Effect of OXT on memory reconsolidation. Error bars represent standard errors of means. (A) Mean percentage of feeders visited belonging to List 1 during recall of List 1 on Day 3. (B) Mean percentage of feeders visited belonging to List 2 during recall of List 1 on Day 3. (*) = P < .05 (**) = P < .01 (***) = P < .001

be attributed to the degree in which the List 1 memory was able to remain stable throughout the experiment. Likewise, since the cue for recall was exclusive to List 1, any unsolicited recollection of List 2, demonstrated by the proportion of List 2 feeders visited, can be attributed to integration of the List 1 and List 2 memories as a result of reconsolidation. Seeing that any integration of memories that occurred was not the result of conscious effort by the rats, feeders visited belonging to List 2 on Day 3 were defined as intrusions. Therefore, variation in the amount of recall or intrusions observed on Day 3 demonstrates differences in the extent to which reconsolidation of the List 1 memory occurred and was used to assess the influence of the administered doses of each drug on reconsolidation.

None of the administered doses of OXT were observed to have an influence on recall (Fig. 5A). The percentage of List 1 feeders visited by rats on Day 3 did not vary significantly between the 0.1 mg/kg (M = 59.72, SD = 1.95, n = 4), 1.0 mg/kg (M = 56.41, SD = 21.27, n = 4), 1.25 mg/kg (M = 58.46, SD = 8.82, n = 7), or 1.5 mg/kg (M = 62.27,

SD = 12.62, *n* = 3) OXT groups. In addition, no variation was found when each of these groups were compared to controls.

Influence on intrusions was found to rely on a dose effect in the OXT groups (Fig. 5B). This effect was characterized by lower doses displaying a higher percentage of List 2 feeders visited by rats on Day 3 and a regression back towards baseline with increasing dosages. Thus, the 0.1 mg/kg OXT group (*M* = 26.01, *SD* = 3.05, *n* = 4) displayed significantly more intrusions than both the saline (t-test, *p* = .013) and combined saline and sesame oil (t-test, *p* = .002) control groups, as well as the neighboring 1.0 mg/kg OXT group (t-test, *p* < .001). No further statistical differences were identified for the 1.0 mg/kg (*M* = 15.40, *SD* = 1.27, *n* = 4), 1.25 mg/kg (*M* = 12.83, *SD* = 5.39, *n* = 7), or 1.5 mg/kg (*M* = 12.82, *SD* = 2.83, *n* = 3) OXT groups when compared to the controls or to each other.

Among the measures analyzed to determine whether the administration of OXT was able to produce effects that influenced reconsolidation, consistent impacts were not able to be identified for any dose. Seeing as memory stability and integration of new information within a memory are characterizations of reciprocal reconsolidation effects, the measures of recall and intrusions that were used to assess these phenomena should exhibit inversed results. Since none of the OXT groups produced any effects on recall, complementary effects were not established. However, this suggests that the doses of OXT administered did not impair the stability of the memory for List 1. Conversely, the dose effect observed on intrusions indicates that information from the memory for List 2 may have been able to become integrated with the memory of List 1. Overall, these results demonstrate that although some effects of OXT were observed in the measures of reconsolidation, they may be dependent on a separate but related construct, rather than reconsolidation alone.

Influence of corticosterone.

The effects of CORT were analyzed in an equivalent manner as OXT in order to identify any influences on reconsolidation. Percent recall and percent intrusions were both evaluated to assess any impact on reconsolidation (Fig. 6A, B).

Some influence on recall was identified in the doses of CORT administered (Fig. 6A). Differences in the percentage of List 1 feeders visited by rats on Day 3 was only

found for the intermediate dose of CORT. Significantly less recall was displayed in the 20 mg/kg CORT group (*M* = 51.84, *SD* = 8.93, *n* = 6) than both the saline (t-test, *p* = .044) and combined saline and sesame oil (t-test, *p* = .025) control groups. This difference was also identified upon comparison to the neighboring 30 mg/kg CORT group (t-test, *p* = .026), but not the 10 mg/kg CORT group (t-test, *p* = .277). Neither the 10 mg/kg (*M* = 59.31, *SD* = 11.40, *n* = 4) nor 30 mg/kg (*M* = 64.05, *SD* = 7.12, *n* = 6) CORT groups displayed additional differences among each other to the controls.

The administered doses of CORT did not appear to have an influence on intrusions (Fig. 6B). The percentages of List 2 feeders visited by rats on Day 3 remained stable across the 10 mg/kg (*M* = 15.22, *SD* = 2.66, *n* = 4), 20 mg/kg (*M* = 16.97, *SD* = 3.87, *n* = 6), and 30 mg/kg (*M* = 12.56, *SD* = 5.23, *n* = 6) CORT groups and among controls.

In identifying whether the administration of CORT was able to produce effects that influenced reconsolidation, no dose was able to establish a stable influence across the measures analyzed. Likewise to the analysis of OXT, complementary effects of reconsolidation were not found in the measures of recall and intrusions, but owing to a lack of differences among intrusions, rather than recall. This suggests that administration

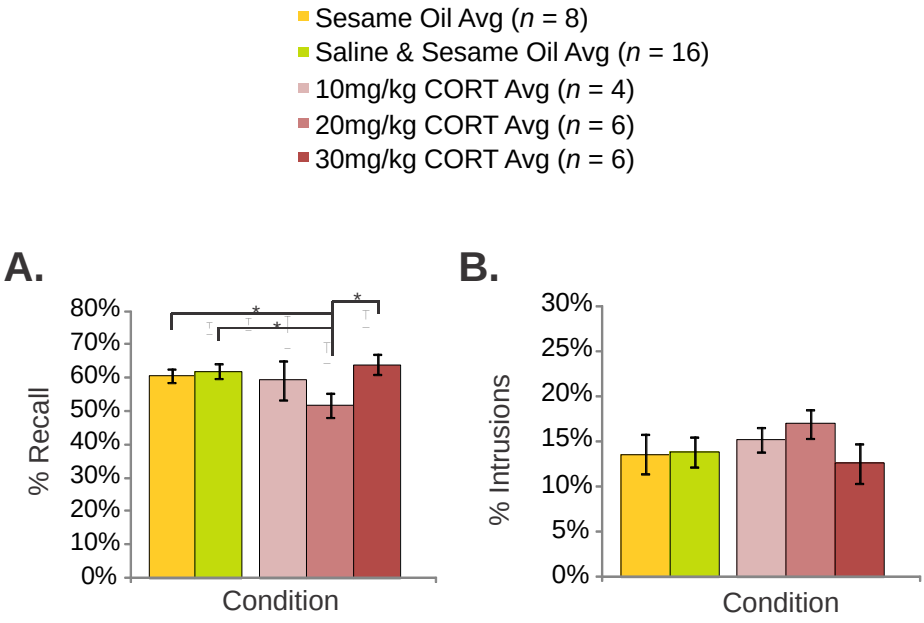

Figure 6. Effect of CORT on memory reconsolidation. Error bars represent standard errors of means. (A) Mean percentage of feeders visited belonging to List 1 during recall of List 1 on Day 3. (B) Mean percentage of feeders visited belonging to List 2 during recall of List 1 on Day 3. (*) = P < .05

of CORT may have had imperative effects on the List 1 memory. All things considered, results were comparable to those seen in OXT and imply that the effects of CORT observed may be dependent on a construct separate, but related to reconsolidation.

Discussion

Research has already demonstrated that when rats are placed in specific spatial contexts involving various sensory cues, including tactile, olfactory, and visual cues, repeated learning in the same context allows the original memory of learned items to be updated with learned concepts from a subsequent memory (Jones et al., 2012). The positive and negative affective states elicited in this study through the anxiolytic and anxiogenic effects of OXT and CORT, respectively, were meant to induce various cues, such as somatic, cognitive, and behavioral variations, that would be able to influence reactivation of previously stored memories and affect reconsolidation similarly to a spatial context. It was predicted that this would cause a significant increase in the amount of intrusions found in the saline and sesame control groups when compared to the OXT and CORT groups, as seen in the Reminder and No Reminder conditions from Jones et al. (2012).

Before analyzing how the results obtained in this experiment compared to the results found in Jones et al. (2012) Experiment 1, the effect of each drug on the rats' ability to perform the experimental task was assessed to determine whether the results on obtained during the reconsolidation assessments were due to the effect of the each drug itself or a drug-induced change in behavior or learning ability. Since all of the control groups were shown to be comparable for each of the measures, only doses of OXT or CORT that displayed an overarching effect that deviated from each control can be said to provide sufficient evidence of an influence on learning or behavior. None of the differences observed between the OXT or CORT groups and controls in the learning and behavioral assessments persisted among all three control groups, with the exception of the 1.5 mg/kg OXT and 10 mg/kg CORT groups for time to prompt the cue delay. Theoretically, although analysis of list learning time was used to assess effects on learning, the behavior exhibited by the rats before the cue delay should persist throughout the entire task. Since a significant increase was not found for both time to prompt the cue delay and list learning time in the 1.5 mg/kg OXT or 10 mg/kg CORT groups, the initial effects on behavior observed may have been the result of the small sample size (n = 3 and n = 4, respectively). However, it is possible that the effects of each drug had subsided by the time the rats prompted the cue delay and no longer posed effects on learning and

behavior. Although this cannot be directly addressed with the analyses performed, rats rarely exceed an hour to complete the task, restricting the amount of time for each drug to wear off. Since peripheral administration of OXT and CORT was shown to produce elevated levels of each drug in relevant brain areas for at least one hour, this is unlikely to be the case (Neumann et al., 2013; Nguyen et al., 2000). Consequently, the peripheral administration of OXT or CORT was not found to affect learning or memory for any dose, allowing direct analysis of the results obtained from the measures of reconsolidation.

The effects of reconsolidation on the memory of List 1 become apparent on Day 3 when List 1 is cued to be recalled. If the List 1 memory was able to become reactivated by factors present on Day 2 and undergo reconsolidation while List 2 is being learned, the memories for these two lists would be able to become associated, causing feeders from List 2 to be recalled along with the List 1 feeders on Day 3. However, if factors present on Day 2 produced sufficient disparities from Day 1 such that the List 1 memory was not able to become reactivated, reconsolidation of List 1 would not occur, or at least would occur to a much lesser degree, allowing it to be more accurately recalled on Day 3. Since the control injections did not contain any active agents, alterations to affective state of the rat should not occur, thus creating a situation resembling that from Day 1 and facilitating reactivation the List 1 memory. Conversely, the administration of OXT and CORT were intended to alter the affective state of the rat relative to Day 1 by producing an increase or decrease in the level of anxiety experienced, respectively, and, for this reason, were hypothesized to discourage the reactivation of the List 1 memory. Therefore, more List 2 intrusions and, consequently, less List 1 recall was expected to be observed among the saline and sesame oil controls than for the various doses of OXT and CORT. The results gathered from this study did not demonstrate the expected trend, but OXT and CORT were observed to have differential effects on reconsolidation. To begin, the effects of OXT and the proposed mechanism in which these effects may have arisen will be discussed, then the effects of CORT will be discussed in a corresponding manner.

OXT did not facilitate better recall of List 1, but a dose effect on the amount of List 2 intrusions was observed. When analyzing recall, no significant differences were found between any two dosages or from any dosage to a control; however, a dose effect in the analysis of intrusions suggested that administration of low doses of OXT led to more List

2 intrusions, with a greater resemblance to controls as the OXT dosage increased. These results seem to indicate that OXT was not able to produce a situation on Day 2 that was sufficiently different enough from Day 1 to prevent reconsolidation, as the recall of List 1 in the OXT groups mirrored the recall exhibited in the controls for which reconsolidation was enabled to occur. Interestingly, low doses of OXT appeared to demonstrate a greater amount of intrusions than would normally occur through reconsolidation, suggesting that OXT, when administered at a low dosage, was either able to strengthen the integration of aspects from the List 2 memory into the List 1 memory during reconsolidation, facilitate the learning of List 2, strengthen the consolidation of the List 2 memory, or any combination of these. Since any ability of OXT to facilitate learning was invalidated by the results from the learning and behavioral assessments, a combination of the ability to enhance integration of the List 1 and List 2 memories during reconsolidation and consolidation of List 2 seems to be the case. Although the effects of OXT or a similar affective state have not previously been examined for reconsolidation, some studies have revealed OXT-dependent memory improvements. For instance, OXT has been linked to improvements in hippocampus-dependent learning and memory during motherhood in mice as well as improved social memory in adult and elderly rats (Tomizawa et al., 2003; Benelli et al., 1995; Popik et al., 1992; Arletti et al., 1995). However, if enhanced consolidation of the List 2 memory was responsible for the effects observed, this would indicate that the List 2 memory needed to be able to become reactivated on Day 3. The ability of spatial context to reactivate a memory and allow it to undergo reconsolidation has already been well established, and indeed, partly inspired this study (Jones et al., 2012). So, because the spatial context was kept constant throughout the entire experiment to determine whether affect alone produced the observed effects, it may have served to reactivate the List 2 memory on Day 3, allowing it to be recalled and contributing to the high rate of intrusions observed. Still, the same context was present on Day 1, allowing it to reactivate the List 1 memory as well. Since the List 1 memory was also cued to be recalled by a blinking light, the List 1 memory was most likely prominent over the List 2 memory for a majority of the task, demonstrating that an exceptional amount of integration of the List 2 memory with the List 1 memory was likely to occur through reconsolidation in addition to enhanced consolidation of the List 2 memory. A summary

of the proposed mechanism in which OXT exerts its influence to produce these results is depicted in Fig. 7A. Overall, administration of low doses of OXT appears to enhance both consolidation and reconsolidation effects, which seems highly plausible as they are likely to rely on similar processes.

CORT, on the other hand, did not affect the amount of List 2 intrusions, but was found to further impair List 1 recall. While no significant differences were found among particular dosages or to controls for the CORT groups in the amount of List 2 intrusions,

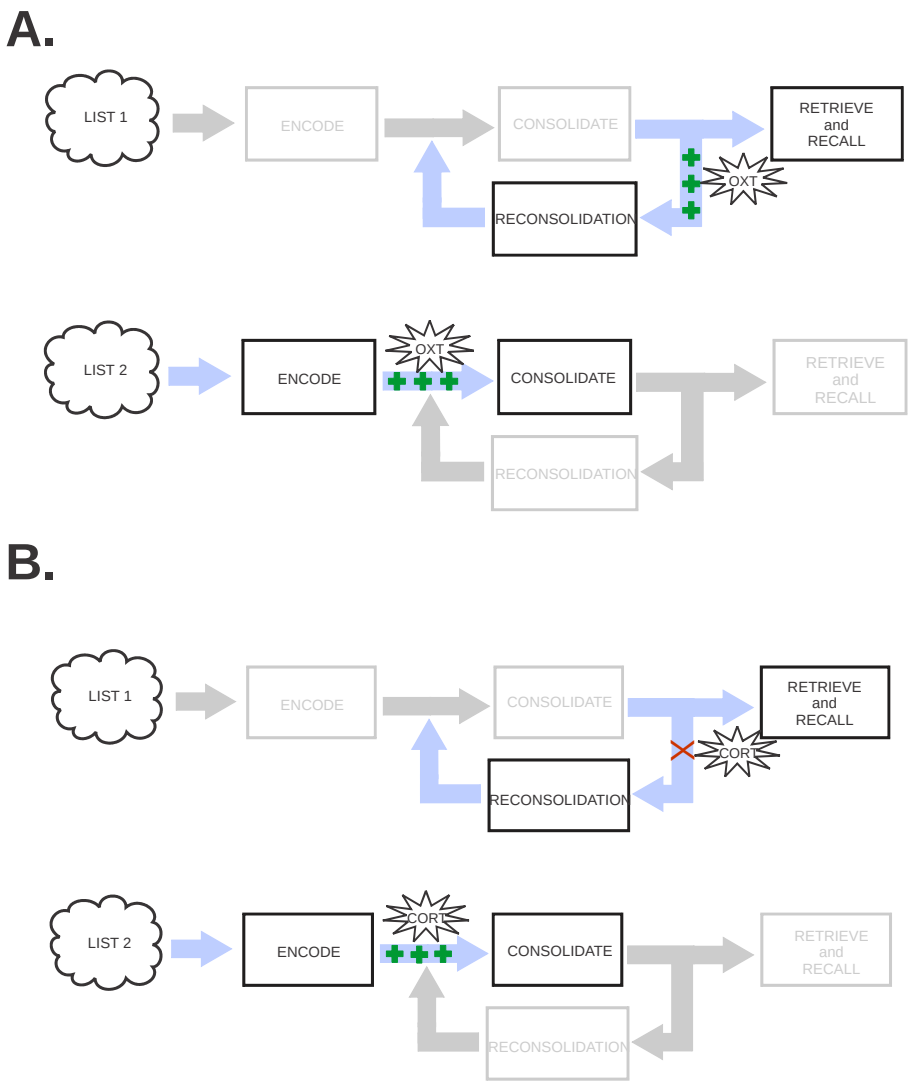

Figure 7. Proposed mechanisms in which OXT and CORT influenced the List 1 and List 2 memories on Day 2 to produce the obtained results. Grayed regions represent memory processes that did not occur on Day 2. Green pluses indicate an enhanced process, red “X”s indicate inhibited processes (A) OXT enhanced both the reconsolidation and consolidation of the List 1 and List 2 memories, respectively. (B) CORT inhibited the reconsolidation of the List 1 memory, but enhanced the consolidation of the List 2 memory.

peripheral administration of 20 mg/kg CORT significantly decreased the amount of List 1 recall compared to both controls and to the 30 mg/kg CORT group. These results seem to suggest that CORT, similarly to OXT, was not able to produce a situation on Day 2 that was sufficiently different enough from Day 1 to prevent reconsolidation, as the amount of List 2 intrusions in the CORT groups mirrored the amount of intrusions exhibited in the controls for which reconsolidation was enabled to occur. Nevertheless, CORT, when administered at an intermediate dose, was able to impede on the stability of the List 1 memory further than would be observed when reconsolidation occurred in normal conditions. A possible mechanism in which this could occur would be for the List 1 memory to become reactivated, but its subsequent reconsolidation to be inhibited by CORT (Fig. 7B). Without sufficient reconsolidation, the List 2 memory would not be able to become adequately incorporated with the memory of List 1 and the List 1 memory itself would lose stability from becoming reactivated, but not being able to restabilize properly through the processes of reconsolidation. Yet, normal amounts of intrusions may have resulted because reactivation of the List 2 memory by the spatial context was able to compensate for decreased integration of the List 1 and List 2 memories. The effects of peripherally administered CORT or stress have not yet been examined for reconsolidation, but have demonstrated differential effects on consolidation and retrieval in both humans (stress) and rats (CORT), with each displaying enhanced consolidation and impaired retrieval (LaBar and Cabeza, 2006; Roozendaal, 2002). Because List 1 and List 2 were at different stages of memory processing, the varying effects of CORT can be used to explain the observed results. For instance, since retrieval has been shown to be impaired under the influence of CORT and requires reactivation of a memory to occur properly, perhaps the underlying cause of this deficiency is due to a reduced reactivation abilities. If so, the decreased reconsolidation proposed to be a result of CORT may be attributed to insufficient reactivation of the List 1 memory. Furthermore, the enhanced consolidation effects of CORT in collection with the reactivating abilities of the spatial context on Day 3 may have served to produce an ample amount of intrusions to resemble the saline and sesame oil controls despite the reduced reconsolidation effects. Ultimately, it is suspected that reduced reactivation effects, but not effects of affective response, from CORT, when administered at an intermediate dose, was able to suppress reconsolidation.

Limitations

This study was able to demonstrate the effects that various dosages of OXT and CORT were able to have on reconsolidation, however, like all studies, some limitations were encountered. For example, most of the groups that were examined contained a relatively small sample size, ranging from 3 to 8 rats, which may have masked potential results or revealed false effects. Nevertheless, these groups were still capable of revealing potential dose effects and provided enough data to at least assume some preliminary conclusions that warrant further investigation. In addition, it was not assured whether the acute administration of OXT or CORT were able to produce the desired affective states at any of the doses as no measures of anxiety were assessed. Despite the lack of confirmation of rats having consciously experienced each desired affective state, as mentioned previously, the endogenous release of OXT and CORT were shown accompany each of these states, so, at the least, application of these drugs was able to replicate the somatic response that would normally occur. However, the results indicate that physiological effects may not be sufficient to represent the experience of an emotional state. Indeed, emotions were chosen to be investigated for their ability to affect both cognition and behavior in addition to their physiological effects. A recent study has reached this same conclusion through analysis of memory for image-context associations under the threat of shock in humans (Bisby and Burgess, 2014). Moreover, the common spatial context throughout the experiment may have served to reactivate the List 1 and List 2 memories unintentionally, biasing the results. The presence of a common spatial context may have allowed the List 1 memory to become reactivated on Day 2 and undergo reconsolidation despite the manipulations to alter the affective state of the rats. In this case, even if being in a different affective state was able inhibit memory reactivation and subsequently reconsolidation, cues from the spatial context would allow it to get reactivated and reconsolidated anyways. Although this may have occurred, this allowed the effects of OXT and CORT on reactivated memories and reconsolidation to be readily assessed, providing meaningful information about their influence on the processes of reconsolidation. Reactivation due to cues from the spatial context may have allowed both list memories to be recalled on Day 3 as well; however, this factor was accounted for in the results.

Future Work

The findings from this study, as well as the limitations detected, propose some further areas of interest for future research. Firstly, in order to account for the influence of spatial context, future researchers may want to look at the impact of pharmacologically induced affective states on reconsolidation with the use of a change in spatial context in addition to the change in affective state. If results demonstrate effects that are amplified when compared to those found with changes in spatial context alone, they can serve to characterize the influence of the change in affective state. Also, it may prove worthy to investigate the effects observed when the affective state is induced on Day 1 and Day 3, rather than Day 2. This alternative may be able to represent any changes that occur with reconsolidation when the memory to be reconsolidated was formed while in a certain emotional state. The idea of state-dependent memories suggests that reactivation on Day 2, when rats would be in a neutral affective state, may necessitate stronger reactivation cues, perhaps more than just the spatial context, to allow the memory to undergo reconsolidation. This would also be able to demonstrate whether memories linked to certain emotional states are easier to reactivate than others. Finally, since it was found that the physiological effects produced through the administration of OXT and CORT may not have been sufficient to represent the experience of an emotional state, manipulations that are able to create situations where these molecules would be released endogenously may be more adequate to investigate the effects of emotion on reconsolidation. This can be achieved by providing a short play period with other rats or putting a rat in a stressful situation before certain days of the experimental task to induce the endogenous release of OXT or CORT, respectively. Overall, these future areas of research would be able to provide more information on the ways in which the experience of an emotion is able to influence memory reconsolidation.

References

Arletti R, Benelli A, Poggioli R, Luppi P, Menozzi B, Bertolini A (1995) Aged rats are still responsive to the antidepressant and memory-improving effects of oxytocin. Neuropeptides 29:177-182.

Ayers LW, Missig G, Schulkin J, Rosen JB (2011) Oxytocin reduces background anxiety in a fear-potentiated startle paradigm: peripheral vs central administration. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 36:2488-2497.

Benelli A, Bertolini A, Poggioli R, Menozzi B, Basaglia R, Arletti R (1995) Polymodal dose-response curve for oxytocin in the social recognition test. Neuropeptides 28:251-255.

Bisby JA, Burgess N (2014) Negative affect impairs associative memory but not item memory. Learning & memory 21:21-27.

Gregus A, Wintink AJ, Davis AC, Kalynchuk LE (2005) Effect of repeated corticosterone injections and restraint stress on anxiety and depression-like behavior in male rats. Behavioural brain research 156:105-114.

Hupbach A, Gomez R, Hardt O, Nadel L (2007) Reconsolidation of episodic memories: a subtle reminder triggers integration of new information. Learning & memory 14:47-53.

Hupbach A, Hardt O, Gomez R, Nadel L (2008) The dynamics of memory: context-dependent updating. Learning & memory 15:574-579.

Jones B, Bukoski E, Nadel L, Fellous JM (2012) Remaking memories: reconsolidation updates positively motivated spatial memory in rats. Learning & memory 19:91-98.

LaBar KS, Cabeza R (2006) Cognitive neuroscience of emotional memory. Nature reviews Neuroscience 7:54-64.

Lee JL, Everitt BJ (2008) Appetitive memory reconsolidation depends upon NMDA receptor-mediated neurotransmission. Neurobiology of learning and memory 90:147-154.

Miller CA, Marshall JF (2005) Molecular substrates for retrieval and reconsolidation of cocaine-associated contextual memory. Neuron 47:873-884.

Morris RG, Inglis J, Ainge JA, Olverman HJ, Tulloch J, Dudai Y, Kelly PA (2006) Memory reconsolidation: sensitivity of spatial memory to inhibition of protein synthesis in dorsal hippocampus during encoding and retrieval. Neuron 50:479-489.

Nadel L, Hupbach A, Gomez R, Newman-Smith K (2012) Memory formation, consolidation and transformation. Neuroscience and biobehavioral reviews 36:1640-1645.

Nader K, Einarsson EO (2010) Memory reconsolidation: an update. Annals of the New York Academy of Sciences 1191:27-41.

Neumann ID (2008) Brain oxytocin: a key regulator of emotional and social behaviours in both females and males. Journal of neuroendocrinology 20:858-865.

Neumann ID, Maloumby R, Beiderbeck DI, Lukas M, Landgraf R (2013) Increased brain and plasma oxytocin after nasal and peripheral administration in rats and mice. Psychoneuroendocrinology 38:1985-1993.

Nguyen KT, Deak T, Will MJ, Hansen MK, Hunsaker BN, Fleshner M, Watkins LR, Maier SF (2000) Timecourse and corticosterone sensitivity of the brain, pituitary, and serum interleukin-1beta protein response to acute stress. Brain research 859:193-201.

Petersson M, Hulting AL, Uvnas-Moberg K (1999) Oxytocin causes a sustained decrease in plasma levels of corticosterone in rats. Neuroscience letters 264:41-44.

Popik P, Vetulani J, van Ree JM (1992) Low doses of oxytocin facilitate social recognition in rats. Psychopharmacology 106:71-74.

Roozendaal B (2002) Stress and memory: opposing effects of glucocorticoids on memory consolidation and memory retrieval. Neurobiology of learning and memory 78:578-595.

Stone EA, Egawa M, McEwen BS (1988) Novel effect of chronic corticosterone treatment on escape behavior in rats. Behavioral and neural biology 50:120-125.

Tomizawa K, Iga N, Lu YF, Moriwaki A, Matsushita M, Li ST, Miyamoto O, Itano T, Matsui H (2003) Oxytocin improves long-lasting spatial memory during motherhood through MAP kinase cascade. Nature neuroscience 6:384-390.

Tronson NC, Taylor JR (2007) Molecular mechanisms of memory reconsolidation. Nature reviews Neuroscience 8:262-275.

Walker MP, Brakefield T, Hobson JA, Stickgold R (2003) Dissociable stages of human memory consolidation and reconsolidation. Nature 425:616-620.

Wang SH, Ostlund SB, Nader K, Balleine BW (2005) Consolidation and reconsolidation of incentive learning in the amygdala. The Journal of neuroscience : the official journal of the Society for Neuroscience 25:830-835.