Received: 15 January 2021 | Revised: 6 August 2021 | Accepted: 28 November 2021

DOI: 10.1002/hipo.23396

WILEY

RESEARCH ARTICLE

Muscarinic (M_1) cholinergic receptor activation within the dorsal hippocampus promotes destabilization of strongly encoded object location memories

Andrew E. Huff | Shelby D. McGraw | Boyer D. Winters

Department of Psychology and Collaborative Neuroscience Program, University of Guelph, Guelph, Ontario, Canada

Correspondence
Andrew E. Huff, Department of Psychology and Collaborative Neuroscience Program, Guelph, Ontario, N1G2W1, Canada.
Email: ehuff@uoguelph.ca

Funding information
Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant, Grant/Award Number: 400176; NSERC Canada Graduate Scholarship Doctorial Award; Ontario Graduate Scholarship

Abstract

Following the initial consolidation process, memories can become reactivated by exposure to a reminder of the original learning event. This can lead to the memory becoming destabilized and vulnerable to disruption or other forms of modification. The memory must then undergo the protein-synthesis dependent process of reconsolidation in order to be retained. However, older and/or stronger memories resist destabilization, but can become labile when reactivated in the presence of salient novelty. We have implicated the neurotransmitter acetylcholine, acting at M_1 muscarinic cholinergic receptors (mAChRs) within perirhinal cortex (PRh), in novelty-induced destabilization of remote object memories. It remains unclear, however, whether mAChRs are involved in destabilization of other forms of memory. We hypothesized that the role of M_1 mAChRs previously demonstrated for PRh-dependent object memory would extend to hippocampus-dependent spatial memory. Using the object location (OL) task, which relies on the dorsal hippocampus (dHPC), we showed that (a) reactivation-dependent reconsolidation of OL memories requires protein synthesis within the dHPC; (b) destabilization of relatively weak OL memories depends on M_1 mAChR activation within the dHPC; (c) salient novelty during reactivation promotes destabilization of resistant strongly encoded OL memories; (d) novelty-induced destabilization of strong OL memories requires activation of mAChRs within the dHPC; and (e) M_1 mAChR activation within the dHPC in the absence of novelty during memory reactivation mimics the effect of novelty, destabilizing strongly encoded OL memories. These results implicate ACh acting at M_1 mAChRs in the destabilization of dHPC-dependent spatial memories, demonstrating generalizability of this cholinergic function beyond memory for object identity. These findings therefore enhance our understanding of the dynamics of long-term memory storage and suggest implications for the treatment of human conditions such as Alzheimer's disease and aging, which are characterized by behavioral and mnemonic inflexibility.

KEYWORDS
acetylcholine, boundary conditions, labilization, recognition, spatial memory

1 | INTRODUCTION

Following the initial learning experience, memories are stabilized through the protein synthesis-dependent process of consolidation (McGaugh, 2000). After this, consolidated memories can become reactivated by re-exposure to cues associated with the original learning experience. This can cause memory destabilization, rendering the memory labile and susceptible to memory updating (Nader & Hardt, 2009). Memory updating can take the form of weakening (e.g., by amnestic drugs such as protein synthesis inhibitors; Nader et al., 2000), strengthening (e.g., by additional training; Lee, 2008), or information integration (e.g., by presenting information immediately post-reactivation; Jardine et al., 2020). The memory must then undergo the protein synthesis-dependent process of reconsolidation in order to restabilize and ensure its persistence within long-term storage (Nader et al., 2000). It is widely thought that this process allows for incorporation of new and updated information to maintain the behavioral relevance of memories (Lee, 2009).

However, not all memories destabilize upon memory reactivation. It has been repeatedly demonstrated that stronger or older memories resist this process (Eisenberg & Dudai, 2004; Suzuki et al., 2004; Winters et al., 2009). Criteria such as age or strength of the memory are known as boundary conditions and influence the likelihood of memory destabilization following reactivation. Interestingly, it has been shown that exposure to salient novelty during memory reactivation can destabilize otherwise resistant object (Winters et al., 2009), fear (Díaz-Mataix et al., 2013; Jarome et al., 2015), and spatial (Morris et al., 2006) memories. This could suggest that older and/or stronger memories, such as those that exert great behavioral control, only destabilize when an obvious opportunity for updating with new information arises.

Recently, we have implicated the neurotransmitter acetylcholine (ACh), which has a critical role in new learning, arousal and attention (Giovannini et al., 2001; Hasselmo, 2006; Hasselmo & Sarter, 2011; Klinkenberg et al., 2011; Sarter & Bruno, 1997), in destabilization and modification of object memories in rats. Indeed, ACh acting at muscarinic cholinergic receptors (mAChRs) appears to be critical for both destabilization of standard object memories, as well as novelty-induced destabilization of older or stronger object memories. This effect relies specifically on the M_1 subtype of mAChRs, and administration of a specific M_1 agonist in the absence of novelty during memory reactivation mimics the effect of novelty, destabilizing otherwise-resistant remote object memories (Stiver et al., 2015; Stiver et al., 2017; Wideman et al., 2021). We have also shown that M_1 mAChRs are critical for memory updating using the post-reactivation object memory modification (PROMM) task, as non-specific, or M_1 subtype specific, mAChR antagonism prevented the integration of contextual and object information (Jardine et al., 2020). Given the contextual nature of the memory modification manipulation used in the PROMM task, it is likely that the hippocampus (HPC) is involved; however, hippocampal treatments were not evaluated in this study. The effects described in the previous reports were observed in tasks evaluating memory for object identity using either systemic or intra-perirhinal cortex (PRh) cholinergic manipulations. It therefore remains unclear whether mAChR activation is important for destabilization of types of memories that rely on other brain structures.

The purpose of the current study was to investigate the generalizability of mAChR involvement in memory destabilization. Accordingly, we assessed whether ACh acting at M_1 mAChRs within the dorsal hippocampus (dHPC) is important for novelty-induced spatial memory destabilization. We hypothesized that this effect is not limited to PRh-based object identity memory and that it extends to destabilization of hippocampus-dependent spatial memory. We used the object location (OL) task, which is dHPC-dependent (Assini et al., 2009; Murai et al., 2007), non-aversive, and has a format that can be easily altered to study memory modification (Kwapis et al., 2019). The current findings provide evidence that acetylcholine acting at M_1 mAChRs is critical for destabilization of relatively weak, as well as strongly encoded object location memories in the hippocampus, similar to what we have previously reported for object identity memory in PRh.

2 | METHODS

2.1 | General procedure

Rats were first implanted with cannulas aimed at the dHPC. Following recovery from surgery, rats were placed on two consecutive days in the open field apparatus one at a time for 5 min/day to habituate them to transportation from the colony room to the testing room, as well as habituate them to the arena. Rats were given mock infusions (infusion cannulas not connected to the pump) prior to being placed into the open field to habituate them to the infusion procedure. Each trial consisted of three phases: the sample phase, the reactivation phase, and the choice phase (Figure 1). During the sample phase, rats were placed into the start box located in the open field. Upon removal of the start box, rats were required to explore objects directly in the sample phase for 30 s; if they did not reach the 30 s criterion, the

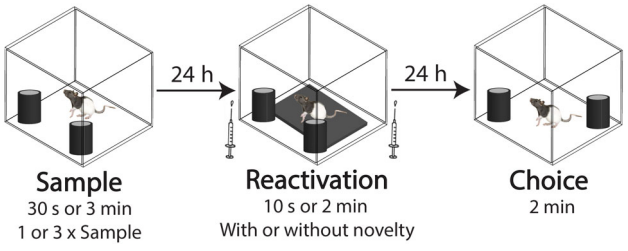

FIGURE 1 Diagram of the object location task with a reactivation phase. This allows for pharmacological manipulations to be made to affect either destabilization (pre-reactivation) or reconsolidation (post-reactivation). In the "standard" version of this task, rats were required to explore objects directly in the sample phase for 30 s; if they did not reach the 30-s criterion, the sample phase ended at 3 min. In the strong memory version, three separate such sample phases were run, separated by 1 h each. In the reactivation phase of the strong memory version, rats were exposed to contextual novelty in the form of a textured floor insert; all other task parameters were the same

sample phase ended at 3 min. Following this, they were removed from the open field and placed back in their cage. Twenty-four hours later, rats completed the reactivation phase, during which they were re-exposed to the same two objects presented in the sample phase. If pre-reactivation infusions were conducted, they were administered either immediately or 15 min prior to the beginning of this phase. Rats were placed into the start box, which was then removed, and rats were required to explore objects directly in the reactivation phase for 10 s; if they did not reach the 10 s criterion, the sample phase ended at 2 min. Following this, they were removed from the open field and underwent their post-reactivation infusion. Twenty-four hours later, rats completed the choice phase, during which they were exposed to the same two objects, but with one moved to a novel location. Rats were placed into the start box, which was then removed, allowing them to explore the two objects for 2 min, following which they were removed from the open field and placed back into their cages.

In the strong memory version of the OL task, rats completed three sample phases (1 h apart) with the same two objects placed in the same two locations. A similar procedure has been previously used to promote the formation of a destabilization-resistant object memory using the SOR task (Winters et al., 2009). Twenty-four hours later, the reactivation session occurred according to the procedure described above, except a textured floor insert was placed in the center of the arena underneath the start box. The choice phase occurred 24 h later according to the previously described procedure.

2.2 | Subjects

Male Long-Evans rats (Charles River, QC) weighing between 250 and 300 g upon arrival were pair-housed in opaque cages (48 × 26 × 20 cm) made of polycarbonate with standard bedding and enrichment, on a reverse light/dark cycle (lights off 8:00–20:00). All behavioral testing took place during the dark phase. Following arrival at the facility, rats were given 1 week to habituate to the facility and researcher handling. For all experiments, water was available ad libitum, except during testing, and rats received 20 g of rodent chow following the conclusion of testing on each day. Rats were food restricted because this enhances exploration (Winters & Bussey, 2005). All procedures followed guidelines established by the Canadian Council on Animal Care and were approved by the Animal Care Committee at the University of Guelph.

2.3 | Surgery

For intra-cranial experiments, 22-gauge indwelling guide cannulas (Plastics 1; HRS Scientific, Quebec) were implanted bilaterally targeting the dHPC. Before and during surgery, rats were anesthetized using isoflurane inhalation anesthetic (Benson Medical Industries, Markham, Ontario). Before beginning the surgery, rats received a subcutaneous injection of Metacam (5 mg/kg) and an intramuscular injection of Baytril (50 mg/kg). Rats were then mounted in a stereotaxic frame (Kopf Instruments, Tujunga, California) using non-puncture ear bars with the incisor bar set at −3.3 mm. The scalp and periosteum were cut (3–4 cm incision) and retracted in order to expose the skull. Target holes were drilled, and guide cannulas were implanted according to the following coordinates, measured relative to bregma (Paxinos & Watson, 2007): anteroposterior −3.8 mm, lateral ±2.5 mm, and dorsoventral −2.5 mm. Cannulas were secured to the skull using four jewelers screws (two anterior to coronal suture, two posterior to lambdoidal suture) as well as dental repair acrylic. Dummy cannulas, 0.36 mm in diameter and cut to be flush with the tip of the guide cannula, were placed into the guide cannulas and remained except during the infusions. Finally, the skin was sutured to close the incision site. Following surgery, rats were allowed to recover with heat for 1–2 h before being placed back into their home cages and were allowed to fully recover for 7 days prior to the initiation of behavioral testing.

2.4 | Drugs

The protein synthesis inhibitor anisomycin [Sigma-Aldrich, Oakville, Ontario; dissolved in 1 N hydrochloric acid (HCl), neutralized to pH = 7.4 with 1 N sodium hydroxide (NaOH) and adjusted to a concentration of 100 μg/μL using 0.9% physiological saline] was administered at a dose of 100 μg/μL. We have used this dose of anisomycin previously to impair memory reconsolidation within PRh and the dHPC (Stiver et al., 2015, 2017; Wideman et al., 2021; Winters et al., 2011). The non-selective mAChR antagonist scopolamine hydrobromide (Sigma-Aldrich, Ontario; dissolved in 0.9% physiological saline) was administered at a dose of 20 μg/μL. We have previously used this dose of scopolamine to prevent memory destabilization within PRh (Stiver et al., 2015). The selective M_1 mAChR antagonist pirenzepine (Sigma-Aldrich, Ontario; dissolved in 0.9% physiological saline) was administered at a concentration of 20 μg/μL. We have previously used this dose of pirenzepine to prevent memory destabilization and memory updating within PRh (Jardine et al., 2020; Stiver et al., 2017). The highly selective M_1 mAChR agonist CDD-0102A (generously donated by Dr. William Messer, University of Toledo; dissolved in 0.9% physiological saline) was administered at a dose of 1 μg/μL. We have previously used this dose of CDD-0102A to facilitate memory destabilization within PRh (Stiver et al., 2017; Wideman et al., 2021). The vehicle control was selected according to the solvent for the corresponding drug.

2.5 | Microinfusion procedure

Intra-cranial microinfusions took place in a separate room adjacent to the behavioral testing room. Rats were gently restrained by the researcher; dummy cannulas were removed and 28-gauge infusion cannulas (cut to extend 1 mm beyond the tip of the guide cannulas) were inserted into the guide cannulas. Bilateral infusions were conducted simultaneously using two 1.0 μL Hamilton syringes driven by a Harvard Apparatus precision syringe pump (Hilliston, Massachusetts), delivering 1.0 μL to each hemisphere over 2 min. Infusion cannulas remained in place for 2 min following the infusion to allow the drug to

diffuse into the tissue. Infusion cannulas were then removed and replaced with sterilized dummy cannulas.

2.6 | Apparatus

Object location experiments were conducted using an open field arena (58.5 × 58.5 × 58.5 cm) made from white, opaque corrugated cardboard. A JVC Everio video camera was mounted on a tripod above the apparatus and used to record behavior for offline rescoring of object exploration. Objects, varying in tactile, visual and material qualities, were affixed to the floor of the apparatus in two adjacent corners using reusable adhesive putty to prevent them from being displaced during each trial. Two copies of a novel, never-before-seen object were used in each trial and were wiped with 50% ethanol prior to the start of the trial. Prior to the initiation of each trial, rats were placed into a tall, bottomless cardboard tube located in the center of the arena that served as a start box. This was to minimize the amount that the rats could explore the objects prior to trial initiation, which occurred when the start box was removed.

2.7 | Experiment 1

Experiment 1 was conducted to assess the effect of protein synthesis inhibition within the dHPC on the reconsolidation of an OL memory. The procedure for experiment 1 was identical to that of the general procedure except rats ($n = 17$) completed three trials in a counterbalanced order, receiving post-reactivation infusions of either vehicle or anisomycin, or anisomycin with no memory reactivation. Rats that were in the no-reactivation condition were transported to the infusion room and underwent their infusion 24 h after their sample to match the timing of the reactivation conditions.

2.8 | Experiment 2

Experiment 2 was conducted to evaluate the effect of blocking mAChRs within the dHPC on the destabilization of OL memories. The procedure for experiment 2 was identical to that of experiment 1 except rats received one of the following drug combinations, with the first listed drug infused 15 min prior to memory reactivation and the second infused immediately following memory reactivation: vehicle/vehicle, vehicle/anisomycin, scopolamine/vehicle, or scopolamine/anisomycin. Fourteen rats from experiment 1 were used for this experiment and completed four trials, one for each drug condition, in a counterbalanced order.

2.9 | Experiment 3

Experiment 3 was conducted to test the effect of M_1 mAChR antagonism within the dHPC on destabilization of OL memories. The procedure for experiment 3 was identical to that of experiment 1 except rats received one of the following drug combinations, with the first infusion immediately prior to memory reactivation and the second infusion immediately following memory reactivation: vehicle/vehicle, vehicle/anisomycin, pirenzepine/vehicle, or pirenzepine/anisomycin. Eleven rats from experiment 1, and five rats from experiment 4, were used for this experiment and completed four trials, one for each drug condition, in a counterbalanced order.

2.10 | Experiment 4

Experiment 4 was conducted to assess whether the presentation of contextual novelty during memory reactivation could destabilize a strongly encoded object location memory. The procedure for this experiment was identical to that of experiment 1, except rats completed three sample phases (1 h apart) to promote the formation of a stronger memory. Contextual novelty was added during memory reactivation by placing a cardboard floor insert (31 × 31 cm), covered with various textured materials (e.g., rubber mat, plastic placemat) in the center of the arena under the start box. Rats ($n = 25$) completed four trials, one with the floor insert and one without the floor insert, for each drug condition (anisomycin and vehicle) in a counterbalanced manner. Rats received one infusion immediately post-reactivation.

2.11 | Experiment 5

Experiment 5 was conducted to evaluate the effect of blocking mAChRs within the dHPC on novelty-induced destabilization of OL memories. The procedure for experiment 5 was identical to that of experiment 4, except rats received one of the following drug combinations, with the first infusion 15 min prior to memory reactivation and the second infusion immediately following memory reactivation: vehicle/vehicle, vehicle/anisomycin, scopolamine/vehicle, or scopolamine/anisomycin. A floor insert was placed under the start box for each reactivation trial. Eight rats from experiment 4 were used for this experiment and completed four trials, one for each drug condition, in a counterbalanced order.

2.12 | Experiment 6

Experiment 6 assessed the effect of M_1 mAChR activation during reactivation of a strongly encoded object location memory. The procedure for experiment 6 was identical to that of experiment 4, except rats received one of the following drug combinations, with the first infusion immediately prior to memory reactivation and the second infusion immediately following memory reactivation: vehicle/vehicle, vehicle/anisomycin, CDD/vehicle, or CDD/anisomycin. Eight rats from experiment 4 were used for this experiment and completed four trials, one for each drug condition, in a counterbalanced order. Rats were not exposed to a textured floor insert on any trials.

2.13 | Histology

Following the conclusion of behavioral testing, rats were anesthetized with intraperitoneal injection of 1.0 mL Euthansol (82 mg/mL; MERK, Intervet Canada Corp., Kirkland, Quebec) and perfused pericardially with PBS followed by 4% neutral buffered formalin. Brains were extracted and stored in 4% formalin in a 3°C fridge for at least 24 h. Brains were then transferred to a solution of 20% sucrose in PBS and left to sink on an orbital shaker. Brains were then sliced with a cryostat in 50 μm slices, and every third slice was mounted onto a gelatin-coated glass microscope slide and thionin stained to verify cannula placement.

2.14 | Data analysis

For OL experiments, two variables were analyzed for each phase of the procedure: total object exploration and phase duration for the sample, total object exploration and phase duration for the reactivation, and total object exploration and discrimination ratio (DR) for the choice. Exploration of an object was defined as the rat directing its nose to the object at a distance of less than 2 cm and/or touching the object with its nose. Turning around, sitting on the object, and chewing the object was not considered exploratory behavior (Winters et al., 2004). Previous findings suggest that the first minute of a choice phase is when rodents are most sensitive to novelty (Dix & Aggleton, 1999; Winters et al., 2011), so choice DR data were analyzed from the first minute only. The DR for the choice was calculated using the following formula [(Object in novel location – object in familiar location)/total object exploration]. For the sample phase, a DR was also calculated, using the same formula based on the to-be-moved object. Total object exploration and phase duration were used as control measures to rule out pre-existing or drug-induced effects on exploratory behavior during the sample, reactivation, and choice phases. These control analyses were always conducted, and are presented in Tables S1–S3, but all were non-significant unless otherwise stated. Since the object locations during the sample are equally novel, a sample DR of approximately 0 is expected, and choice and sample DRs were compared for each drug condition using paired-sample *t*-tests as an analysis of "chance" performance in the choice phase; a significant difference between the sample and choice DRs indicates discrimination between the object in the novel location and the object in the familiar location. Choice DR was also analyzed using repeated measures ANOVAs. Post-hoc analyses were conducted to assess significant effects of drug condition using paired-sample *t*-tests with a Bonferroni correction. The alpha level was set at 0.05 for all non-corrected analyses.

3 | RESULTS

3.1 | Histology

All rats that were included in final behavioral analyses had bilateral cannulae with infusion needle tips terminating within the dHPC (Figure 2). Placements were consistently located between −3.14 and −3.60 mm relative to bregma.

3.2 | Experiment 1

Experiment 1 assessed the necessity of protein synthesis within the dHPC for reconsolidation of an object location memory. Immediate post-reactivation intra-dHPC microinfusions of anisomycin impaired reconsolidation of reactivated OL memories (Figure 3). A repeated measures ANOVA on the choice phase DR data indicated a significant effect of drug [$F(2, 32) = 15.416$, $p < .001$, partial $\eta^2 = 0.491$]. Post-hoc paired-samples *t*-tests revealed that rats had a significantly lower choice DR when they were infused with anisomycin, compared with when they were infused with vehicle [$t(16) = 5.901$, $p < .001$] or did not undergo memory reactivation [$t(16) = 3.825$, $p = .001$]. Consistent with this, paired-samples *t*-tests revealed that sample DR was significantly different from choice DR in the vehicle condition [$t(16) = 6.411$, $p < .001$] and the no-reactivation condition [$t(16) = 3.970$, $p = .001$], but not in the anisomycin condition [$t(19) = -0.683$, $p = .505$], where there was no evidence that rats successfully discriminated between the object in the novel location and the object in the familiar location.

3.3 | Experiment 2

Experiment 2 assessed the necessity of mAChRs within the dHPC for destabilization of an object location memory. Pre-reactivation intra-dHPC microinfusions of scopolamine prevented memory destabilization, protecting the memory against the impairing effects of post-reactivation intra-dHPC anisomycin (Figure 4). A repeated measures ANOVA on the choice phase DR data indicated a significant effect of drug [$F(3, 39) = 7.542$, $p < .001$, partial $\eta^2 = 0.37$]. Post-hoc paired-samples *t*-tests revealed that rats had a significantly lower choice DR when they were infused with vehicle pre-reactivation and anisomycin post-reactivation, compared with all other drug conditions (vehicle/vehicle: [$t(13) = 3.853$, $p = .002$]; scopolamine/vehicle: [$t(13) = 3.494$, $p = .004$]; scopolamine/anisomycin [$t(13) = 3.650$, $p = .003$]). Consistent with this, paired-samples *t*-tests revealed that sample DR was significantly different from choice DR in all drug conditions (vehicle/vehicle: [$t(13) = 2.395$, $p = .032$]; scopolamine/vehicle: [$t(13) = 2.828$, $p = .014$]; scopolamine/anisomycin [$t(13) = 2.784$, $p = .015$]), except in the vehicle/anisomycin condition [$t(13) = -1.747$, $p = .104$], where there was no evidence that rats successfully discriminated between the object in the novel location and the object in the familiar location. A repeated measures ANOVA on reactivation phase duration data indicated a significant effect of drug [$F(3, 39) = 2.945$, $p = .045$, partial $\eta^2 = 0.19$]. Post-hoc paired sample *t*-tests revealed that rats infused with scopolamine pre-reactivation and anisomycin post-reactivation took significantly longer [$t(13) = 2.867$, $p = .013$] to reach the exploration criteria than those infused with vehicle pre-reactivation and anisomycin post-reactivation. All other control analyses were non-significant.

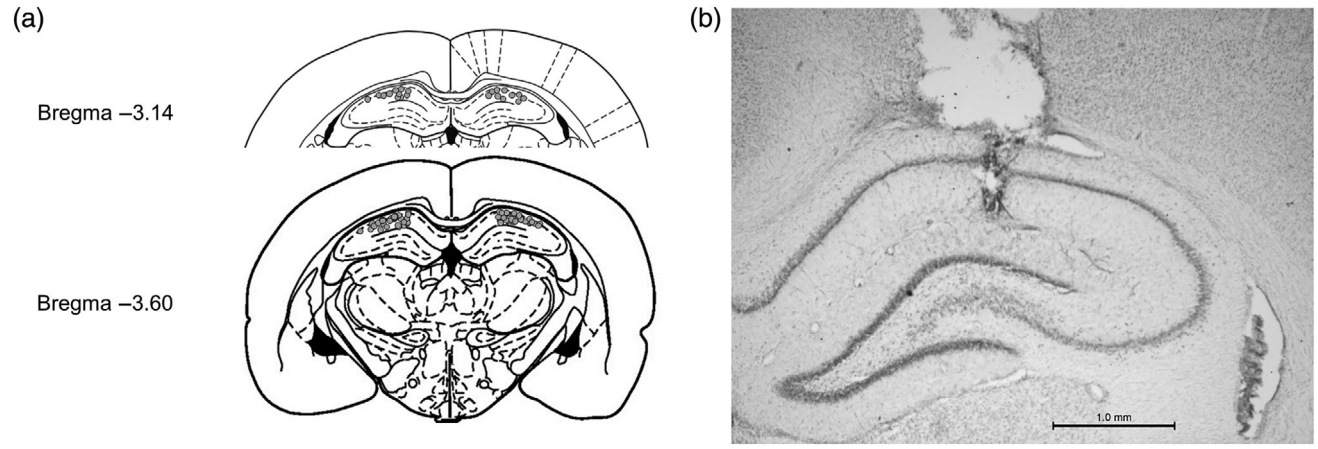

FIGURE 2 Cannula placements in the dorsal hippocampus. (a) Schematic of infusion tip placements in the dorsal hippocampus from all rats used in behavioral experiments. (b) Photomicrograph illustrating cannula and infusion needle tract terminating within the dHPC

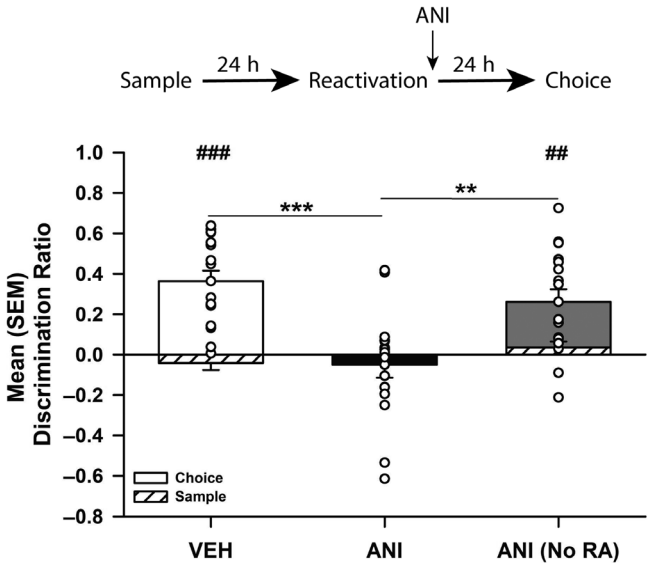

FIGURE 3 In experiment 1, immediate post-reactivation administration of anisomycin (ANI) into the dorsal hippocampus prevented reconsolidation of an object location memory. This effect was eliminated when the reactivation phase was omitted, suggesting that reconsolidation of object location memories is both protein synthesis-dependent within the dHPC, and reactivation-dependent. Values are displayed as average discrimination ratio ± SE. * indicates a significant drug group choice discrimination ratio comparison; # indicates a significant sample-choice comparison for that drug group. * or # indicates $p < .05$; ** or ## indicates $p < .01$; *** or ### indicates $p < .001$. $N = 17$; VEH, vehicle

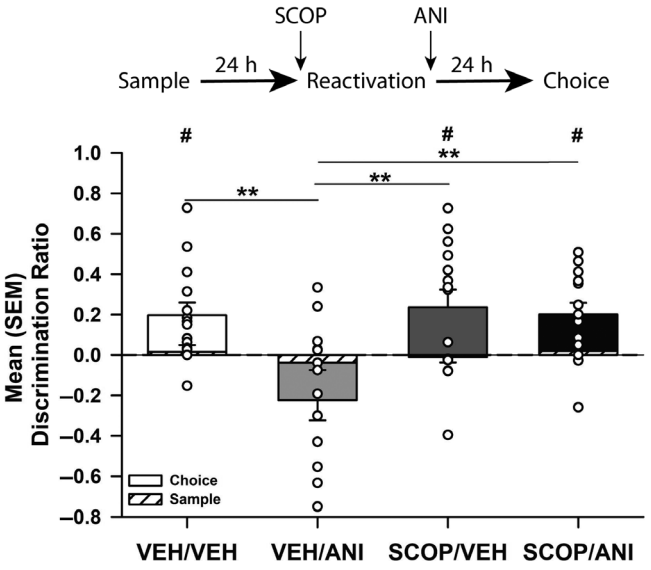

FIGURE 4 In experiment 2, pre-reactivation intra-dorsal hippocampus non-selective muscarinic cholinergic receptor antagonism (scopolamine; SCOP) prevented anisomycin (ANI)-induced reconsolidation impairment, suggesting that destabilization of object location memories relies on muscarinic cholinergic receptors within the dorsal hippocampus. Values are displayed as average discrimination ratio ± SE. * indicates a significant between drug group choice discrimination ratio comparison; # indicates a significant sample-choice comparison for that drug group. * or # indicates $p < .05$; ** or ## indicates $p < .01$; *** or ### indicates $p < .001$. $N = 14$. VEH, vehicle

3.4 | Experiment 3

Experiment 3 assessed the necessity of M_1 mAChRs within the dHPC for destabilization of an object location memory. Pre-reactivation intra-dHPC microinfusions of the M_1 mAChR antagonist pirenzepine prevented memory destabilization, protecting the memory against the impairing effects of post-reactivation intra-dHPC anisomycin (Figure 5). A repeated measures ANOVA on the choice phase DR data indicated a significant effect of drug [$F(3, 45) = 7.331$, $p < .001$, partial $\eta^2 = 0.33$]. Post-hoc paired-samples t-tests revealed that rats had a significantly higher choice DR when they were infused with pirenzepine pre-reactivation and anisomycin post-reactivation [$t(15) = 3.817$, $p = .002$], pirenzepine pre-reactivation and vehicle post-reactivation [$t(15) = 4.007$, $p = .001$], or vehicle pre-reactivation and vehicle post-reactivation [$t(15) = 4.051$, $p = .001$] compared with

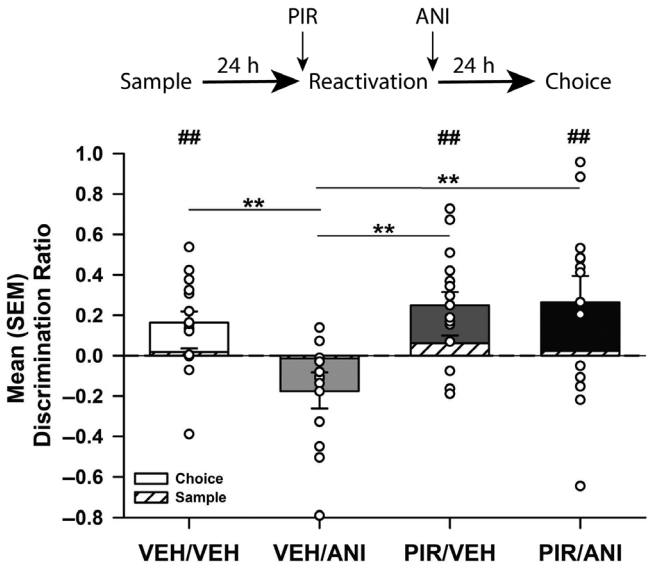

FIGURE 5 In experiment 3, pre-reactivation intra-dorsal hippocampus selective M_1 muscarinic cholinergic receptor antagonism (pirenzepine; PIR) prevented the anisomycin (ANI)-induced reconsolidation impairment, suggesting that destabilization of object location memories relies on M_1 muscarinic cholinergic receptors within the dorsal hippocampus. Values are displayed as average discrimination ratio ± SE. * indicates a significant between drug group choice discrimination ratio comparison; # indicates a significant sample-choice comparison for that drug group. * or # indicates $p < .05$; ** or ## indicates $p < .01$; *** or ### indicates $p < .001$. $N = 16$. VEH, vehicle

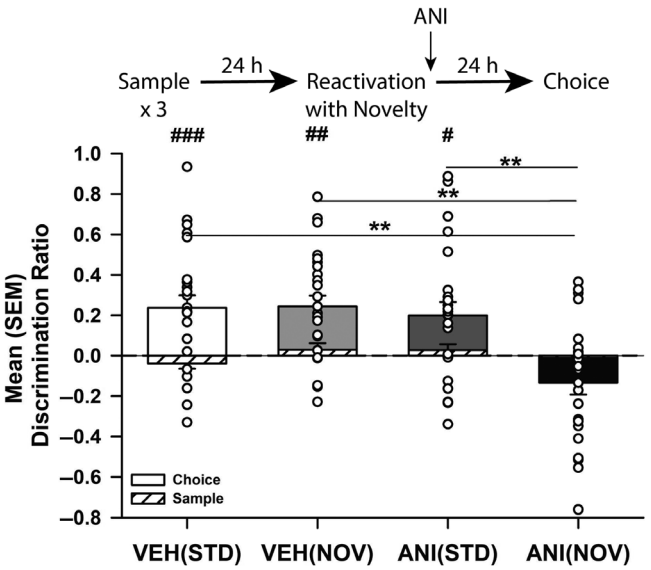

FIGURE 6 In experiment 4, post-reactivation intra-dorsal hippocampus anisomycin (ANI) impaired memory reconsolidation, but only when rats were exposed to novelty during memory reactivation, suggesting that novelty is required to destabilize strongly encoded object location memories. Values are displayed as average discrimination ratio ± SE. * indicates a significant between drug group choice discrimination ratio comparison; # indicates a significant sample-choice comparison for that drug group. * or # indicates $p < .05$; ** or ## indicates $p < .01$; *** or ### indicates $p < .001$. $N = 25$. VEH, vehicle

when they received vehicle pre-reactivation and anisomycin post-reactivation. Paired-samples *t*-tests revealed that sample DR was significantly different from choice DR in all drug conditions (pirenzepine/anisomycin [$t(15) = 2.198$, $p = .044$]; pirenzepine/vehicle [$t(15) = 2.467$, $p = .026$]; and vehicle/vehicle [$t(15) = 2.465$, $p = .026$]), except in the vehicle/anisomycin condition [$t(15) = -1.758$, $p = .099$], where there was no evidence that rats successfully discriminated between the object in the novel location and the object in the familiar location.

3.5 | Experiment 4

Experiment 4 assessed the effect of exposure to novelty during reactivation of a strongly encoded object location memory. Post-reactivation intra-dHPC microinfusions of anisomycin prevented memory reconsolidation, but only when rats were exposed to contextual novelty in the form of a floor insert during memory reactivation (Figure 6). A repeated measures ANOVA on the choice phase DR data indicated a significant drug by reactivation type interaction [$F(1, 24) = 6.203$, $p = .020$, partial $\eta^2 = 0.21$], a significant main effect of drug [$F(1, 24) = 12.372$, $p = .002$, partial $\eta^2 = 0.34$], and a significant main effect of reactivation type [$F(1, 24) = 6.537$, $p = .017$, partial $\eta^2 = 0.21$]. Post-hoc paired-samples *t*-tests revealed that rats had a significantly higher choice DR when they were infused with vehicle post-reactivation with no novelty [$t(24) = 4.852$, $p < .001$], vehicle post-reactivation with novelty [$t(24) = 4.864$, $p < .001$] or anisomycin post-reactivation with no novelty [$t(24) = 3.228$, $p = .004$], compared with when they received vehicle pre-reactivation and anisomycin post-reactivation. Paired-samples *t*-tests revealed that sample DR was significantly different from choice DR in all drug conditions (vehicle × no novelty [$t(24) = 4.002$, $p = .001$]; vehicle × novelty [$t(24) = 2.968$, $p = .007$]; and anisomycin × no novelty [$t(24) = 2.424$, $p = .023$]), except in the anisomycin x novelty condition [$t(24) = -2.029$, $p = .054$], where there was no evidence that rats successfully discriminated between the object in the novel location and the object in the familiar location.

A repeated measures ANOVA on the reactivation phase duration DR data indicated a significant main effect of reactivation type [$F(1, 24) = 64.115$, $p < .001$, partial $\eta^2 = 0.73$]. Post-hoc paired sample *t*-tests revealed that rats had a significantly longer reactivation phase duration when they were infused with vehicle post-reactivation with novelty compared with when they were in the anisomycin × no novelty [$t(24) = 5.384$, $p < .001$] or the vehicle × no novelty conditions [$t(24) = 4.918$, $p < .001$] and a significantly longer reactivation phase duration when they were infused with anisomycin post-reactivation with novelty compared with when they were in the anisomycin × no novelty [$t(24) = 3.006$, $p = .006$] or the vehicle × no novelty conditions [$t(24) = 5.319$, $p < .001$].

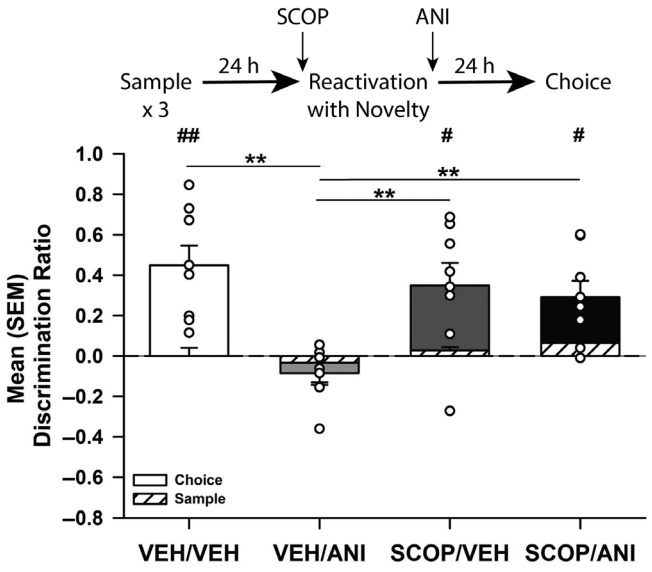

FIGURE 7 In experiment 5, pre-reactivation intra-dorsal hippocampus non-selective muscarinic cholinergic receptor antagonism (scopolamine; SCOP) prevented the anisomycin (ANI)-induced reconsolidation impairment, suggesting that novelty-induced destabilization of strongly encoded object location memories relies on muscarinic cholinergic receptors within the dHPC. Values are displayed as average discrimination ratio ± SE. * indicates a significant between drug group choice discrimination ratio comparison; # indicates a significant sample-choice comparison for that drug group. * or # indicates $p < .05$; ** or ## indicates $p < .01$; *** or ### indicates $p < .001$. $N = 8$. VEH, vehicle

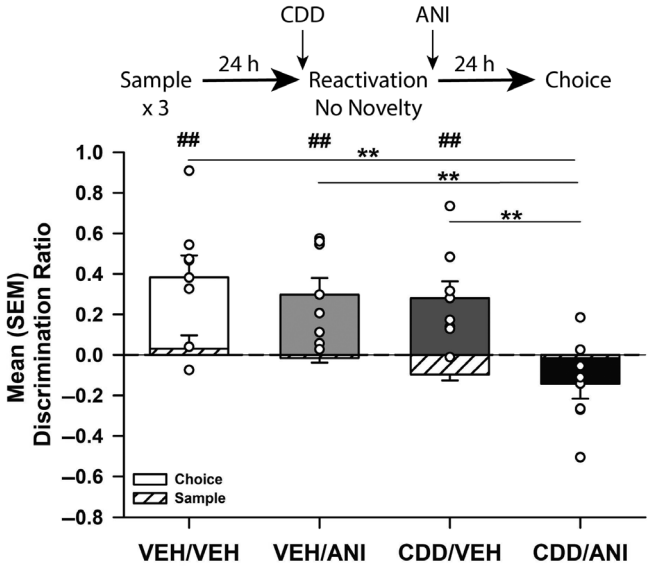

FIGURE 8 In experiment 6, pre-reactivation intra-dorsal hippocampus M_1 selective muscarinic cholinergic receptor activation (with CDD-0102A; CDD) induced destabilization, allowing for the anisomycin (ANI)-induced reconsolidation impairment, suggesting that M_1 receptor activation mimics the effect of novelty during memory reactivation. Values are displayed as average discrimination ratio ± SE. * indicates a significant between drug group choice discrimination ratio comparison; # indicates a significant sample-choice comparison for that drug group. * or # indicates $p < .05$; ** or ## indicates $p < .01$; *** or ### indicates $p < .001$. $N = 8$. VEH, vehicle

3.6 | Experiment 5

Experiment 5 assessed the necessity of mAChRs within the dHPC for novelty-induced destabilization of strongly encoded object location memory. Pre-reactivation intra-dHPC microinfusions of scopolamine prevented novelty-induced destabilization, protecting the memory against the impairing effects of post-reactivation intra-dHPC anisomycin (Figure 7). A repeated measures ANOVA on the choice phase DR data indicated a significant effect of drug [$F(3, 21) = 6.705$, $p = .002$, partial $\eta^2 = 0.49$]. Post-hoc paired-samples t-tests revealed that rats had a significantly lower choice DR when they were infused with vehicle pre-reactivation and anisomycin post-reactivation, compared with all other drug conditions (vehicle/vehicle: [$t(7) = -4.765$, $p = .002$]; scopolamine/vehicle: [$t(7) = -3.693$, $p = .008$]; scopolamine/anisomycin [$t(7) = -4.007$, $p = .005$]). Consistent with this, paired-samples t-tests revealed that sample DR was significantly different from choice DR in all drug conditions (vehicle/vehicle: [$t(7) = 6.049$, $p = .001$]; scopolamine/vehicle: [$t(7) = 3.023$, $p = .019$]; scopolamine/anisomycin [$t(7) = 3.137$, $p = .016$]), except in the vehicle/anisomycin condition [$t(7) = -0.837$, $p = .430$], where there was no evidence that rats successfully discriminated between the object in the novel location and the object in the familiar location.

3.7 | Experiment 6

Experiment 6 assessed the effect of M_1 mAChR activation during reactivation of a strongly encoded object location memory. Pre-reactivation intra-dHPC microinfusions of the M_1 specific agonist CDD induced memory destabilization, rendering the strongly encoded memory vulnerable to the amnestic effects of anisomycin, even without the explicit presence of novelty during reactivation (Figure 8). A repeated measures ANOVA on the choice phase DR data indicated a significant effect of drug [$F(3, 21) = 7.605$, $p = .001$, partial $\eta^2 = 0.52$]. Post-hoc paired-samples t-tests revealed that rats had a significantly higher choice DR when they were infused with CDD pre-reactivation and vehicle post-reactivation [$t(7) = 3.301$, $p = .013$], vehicle pre-reactivation and anisomycin post-reactivation [$t(7) = 3.268$, $p = .014$], or vehicle pre-reactivation and vehicle post-reactivation [$t(7) = 4.412$, $p = .003$] compared with when they received CDD pre-reactivation and anisomycin post-reactivation. Paired sample t-tests revealed that sample DR was significantly different from choice DR in all drug conditions (CDD/vehicle [$t(7) = 3.922$, $p = .006$]; vehicle/anisomycin [$t(7) = 4.159$, $p = .004$]; and vehicle/vehicle [$t(7) = 3.628$, $p = .008$]), except in the CDD/anisomycin condition [$t(7) = -1.636$, $p = .146$], where there was no evidence that rats successfully discriminated between the object in the novel location and the object in the familiar location.

4 | DISCUSSION

Here, we have presented data that support the idea that ACh acting at M_1 mAChRs within the dHPC is critical for spatial memory

destabilization, similar to what our group has previously demonstrated for M_1 mAChRs within PRh for object identity memory. First, we showed that post-reactivation anisomycin administration into the dHPC results in memory impairments when rats were tested 24 h later. Similar to previous findings (Nader et al., 2000; Winters et al., 2009), when the reactivation phase was omitted, anisomycin had no effect, suggesting that this effect was reactivation-dependent. While it has been shown that reconsolidation of an updated OL memory using modified OL tasks depends upon protein synthesis within the dHPC (Choi et al., 2010; Kwapis et al., 2019), it was still critical that we show that reconsolidation of an unmodified OL memory is protein synthesis-dependent within the dHPC, which is consistent with findings from the fear conditioning literature (Debiec et al., 2002). Having shown the typical anisomycin-induced reconsolidation impairment in the OL task, we were then able to assess the generalizability of previous findings regarding the role of mAChRs in the destabilization of object memories. Extending our previous findings for object identity memory destabilization (Stiver et al., 2015, 2017), as well as for information integration in the PROMM task (Jardine et al., 2020), both non-specific (scopolamine) or M_1 specific (pirenzepine) mAChR antagonism blocked spatial memory destabilization, protecting the OL memory from the typical anisomycin-induced reconsolidation impairment. In both of these experiments, we replicated the intra-dHPC anisomycin-induced reconsolidation impairment.

It is important to note that rats in the scopolamine/anisomycin condition did take significantly longer to reach the exploration criteria in the reactivation phase than those in the vehicle/anisomycin condition. However, they did not differ in terms of total object exploration during the reactivation phase, so it is unlikely that the memory was not sufficiently destabilized due to a lack of exploration for anisomycin to have an effect in the scopolamine/anisomycin condition. In addition, scopolamine typically does not impair locomotion (Ossenkopp et al., 1986; Watanabe & Shimizu, 1989) nor did we observe any differences in exploration between the scopolamine/vehicle and vehicle/anisomycin conditions.

In experiments 4–6, we assessed whether novelty could destabilize an otherwise-resistant OL memory, and whether mAChRs within the dHPC were involved in this novelty-induced destabilization. We first showed that giving rats three sample phases instead of one promoted the formation of a destabilization-resistant memory; however, similar to our previous results with object identity memory in PRh (Stiver et al., 2015, 2017; Wideman et al., 2021; Winters et al., 2009, 2011), when rats were exposed to explicit contextual novelty during memory reactivation, post-reactivation intra-dHPC anisomycin impaired spatial memory reconsolidation. When anisomycin was given following reactivation with no novelty, it had no observable effect on memory. This suggests that exposure to salient contextual novelty was required during memory reactivation to initiate destabilization of the strongly encoded object location memory, which is in line with the postulated role of memory reconsolidation in flexibly maintaining the behavioral relevance of memories. In experiment 4, we observed that when rats were reactivated in the presence of contextual novelty, they took significantly longer to reach the exploration criteria (10 s of object exploration) during the reactivation phase. However, their total exploration was not significantly different from when they were reactivated without novelty, and we have previously observed this effect (Winters et al., 2009). This suggests that the rats spent time investigating the floor insert, suggesting that it was recognized as novel, which was the purpose of introducing it.

We then showed that pre-reactivation administration of scopolamine into the dHPC blocked this novelty-induced destabilization, preventing anisomycin from impairing memory reconsolidation. When scopolamine was not given pre-reactivation, we replicated the anisomycin-induced reconsolidation impairment following exposure to novelty during memory reactivation. Lastly, we demonstrated that administration of an M_1 specific agonist prior to reactivation without contextual novelty promoted destabilization of a strongly encoded OL memory, rendering the memory vulnerable to the impairing effects of anisomycin. Stiver et al. (2015, 2017) previously reported that mAChRs within PRh were required for novelty-induced destabilization of remote object memories, and that activation of M_1 mAChRs within PRh replicated the effects of novelty during reactivation, promoting destabilization of older object memories. Together with the current study's findings, this suggests that the destabilization of otherwise-resistant memories can be promoted by activation of M_1 mAChRs within at least two brain regions and for two different types of memory. While it is possible that this may not extend to other brain regions, or other forms of memories, it does suggest that novelty-related mAChR stimulation could be a general mechanism for destabilization (and updating) of otherwise-resistant memories.

In both experiment 2 and experiment 5, pre-reactivation microinfusions of scopolamine had no effect on the ability of rats to discriminate between the object in the sampled location and an object in a novel location when it was not followed by anisomycin. Scopolamine has been shown to have amnestic effects when administered prior to learning but typically does not block consolidation or retrieval (Warburton et al., 2003; Winters et al., 2006). Therefore, it is unlikely that the failure of anisomycin to block reconsolidation in the scopolamine/anisomycin condition is due to a failure to retrieve the memory during reactivation. Even if pre-reactivation scopolamine did block retrieval, there is evidence to suggest that retrieval and destabilization are dissociable processes, at least within PRh (Balderas et al., 2013), suggesting a specific effect of scopolamine on destabilization. In addition, Stiver et al. (2015) showed that pre-reactivation scopolamine did not prevent retrieval when the reactivation phase included a novel object along with the familiar. Recently, Krawczyk et al. (2021) reported that post-reactivation infusion of scopolamine or pirenzepine into the dHPC impaired reconsolidation of an inhibitory avoidance memory which was still evident 21 days after the memory test. They have previously reported that blockade of $\alpha 7$ nicotinic receptors within the dHPC also impaired reconsolidation of an inhibitory avoidance memory (Boccia et al., 2010); thus it appears that ACh acting at both nAChRs and mAChRs is necessary for reconsolidation of inhibitory avoidance memories, which suggests that ACh may be differentially involved in the storage processes for these different forms of memory. Future work will be needed to resolve this question.

Although the mean discrimination ratio was below zero in a few experiments, the sample-to-choice comparison for the VEH/ANI condition was not significant for any of these. Thus, post-reactivation intra-dHPC anisomycin did not significantly bias rats toward the familiar object location. In addition, as all experiments were completed in a counterbalanced within-subjects design, it is unlikely that there were any lingering effects of anisomycin on behavior or memory during the subsequent trial(s). There is also work indicating that anisomycin has effects in addition to inhibition of protein synthesis (Dubue et al., 2015; Scavuzzo et al., 2019; Sharma et al., 2012), showing repeatedly that anisomycin can transiently reduce neural activity within the HPC, at comparable doses to what we and others have used to impair memory reconsolidation. While the consensus in the literature is that memory reconsolidation is protein synthesis-dependent, this is largely based on findings utilizing anisomycin. However, based on work from the Dickson group (Dubue et al., 2015; Scavuzzo et al., 2019; Sharma et al., 2012), for example, it is possible that anisomycin disrupts memory reconsolidation by some other mechanism. In the current study, our focus was on how mAChR activity affects memory destabilization. Whether anisomycin impairs memory reconsolidation by blocking protein synthesis or through a different mechanism, when scopolamine or pirenzepine were given pre-reactivation, the memory impairment did not occur. Thus, critically for the aims of the current study, these findings are consistent with the interpretation that mAChR antagonism prevents memory destabilization, thereby negating the amnestic effects of anisomycin, regardless of the specific mechanism of the latter.

The M_1 mAChR was of particular interest for the current study, as we have previously reported that M_1, but not M_2, mAChRs are important for novelty-induced destabilization of object memories (Stiver et al., 2017). Importantly, M_1 mAChRs are linked to the ubiquitin-proteasome system (UPS), which is thought to be responsible for the degradation of synaptic structural proteins that are involved in the stabilization and maintenance of the memory engram (Jarome et al., 2011; Lee et al., 2008). This mechanism has been posited as a physiological correlate of memory destabilization. Following activation of M_1 mAChRs on the cell membrane, phospholipase C (PLC) is activated, which is linked to M_1 mAChRs via G-proteins (Horowitz et al., 2005). PLC stimulates the production of the second messenger inositol 1,4,5-triphosphate (IP_3), which binds to its receptor (IP_3R) to induce the release of calcium from within the endoplasmic reticulum (Felder, 1995). This increase in intracellular calcium can lead to the activation of CaMKII, which is an important regulator of the activity of the UPS (Jarome & Helmstetter, 2013). Our group has shown that while remote object memories can be destabilized by activating M_1 mAChRs, this can be prevented by blocking IP_3Rs, inhibiting CaMKII, or inhibiting the UPS (Stiver et al., 2017; Wideman et al., 2021). It is likely that this mechanism is also involved in the destabilization of dHPC-dependent memories, as the current findings implicate M_1 mAChRs in the destabilization of resistant memories, and other reports implicate the UPS in the destabilization of dHPC-dependent memories (Lee, 2008; Lee et al., 2008).

Thus, the present study builds on previous findings from our group implicating PRh M_1 mAChRs in destabilization and modification of object memories (Jardine et al., 2020; Stiver et al., 2015, 2017; Wideman et al., 2021), showing that destabilization of relatively weak object location memories, as well as novelty-induced destabilization of strongly encoded object location memories, depends on mAChRs within the dHPC. Importantly, we also show that activation of M_1 mAChRs within the dHPC drives destabilization of otherwise-resistant object location memories, even without the explicit presence of contextual novelty during memory reactivation. This suggests that the mAChR-dependent mechanism is generalizable to at least two brain regions and two types of memories and may be a general mechanism for novelty-induced memory destabilization. These results also have important implications for forms of memory updating beyond reconsolidation disruption. We have previously demonstrated that integration of contextual information with object identity memories is M_1 receptor dependent in PRh (Jardine et al., 2020), and here we provide evidence that destabilization of spatial memories requires M1 receptor activity in the HPC. It is, therefore, possible that integration of new information into HPC-dependent spatial memories also relies on cholinergic signaling at M_1 receptors. Utilizing spatial memory information integration tasks such as the objects in updated locations task (Kwapis et al., 2019), or a HPC-dependent version of the PROMM task (Jardine et al., 2020), should help to address this unanswered question. This research suggests implications for our understanding of the dynamics of long-term memory storage, and the potential treatment of behavioral and cognitive inflexibility that can occur in aging, dementia, and other disorders. Given that M_1 mAChR activation appears to promote the lability, or flexibility, of otherwise-resistant memories, this could be a pharmacological target exploited in conjunction with cognitive-behavioral therapy to aid in the treatment of disorders characterized by strongly encoded maladaptive memories, such as phobias and post-traumatic stress disorder.

ACKNOWLEDGMENTS

Thank you to Dr. William Messer (University of Toledo, OH, USA) for generously donating the CDD-0102A. Thank you to Herleena Verraich and Jessica Kespe for their assistance with running pilot experiments.

This research was funded by a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant to B.D.W. (400176), as well as an NSERC Canada Graduate Scholarship Doctoral Award and an Ontario Graduate Scholarship to A.E.H.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The behavioural data presented in the study are available in the supplementary material (Tables S1–S3).

ORCID

Andrew E. Huff https://orcid.org/0000-0001-6876-9164

REFERENCES

Assini, F. L., Duzzioni, M., & Takahashi, R. N. (2009). Object location memory in mice: Pharmacological validation and further evidence of hippocampal CA1 participation. *Behavioural Brain Research*, *204*, 206–211. https://doi.org/10.1016/j.bbr.2009.06.005

Balderas, I., Rodriguez-Ortiz, C. J., & Bermudez-Rattoni, F. (2013). Retrieval and reconsolidation of object recognition memory are independent processes in the perirhinal cortex. *Neuroscience*, *253*, 398–405. https://doi.org/10.1016/j.neuroscience.2013.09.001

Boccia, M. M., Blake, M. G., Krawczyk, M. C., & Baratti, C. M. (2010). Hippocampal Alpha7 nicotinic receptors modulate memory consolidation of an inhibitory avoidance task in mice. *Neuroscience*, *171*, 531–543. https://doi.org/10.1016/j.neuroscience.2010.08.027

Choi, J.-H., Kim, J.-E., & Kaang, B.-K. (2010). Protein synthesis and degradation are required for the incorporation of modified information into the pre-existing object-location memory. *Molecular Brain*, *3*, 1. https://doi.org/10.1186/1756-6606-3-1

Debiec, J., Ledoux, J. E., & Nader, K. (2002). Cellular and systems reconsolidation in the hippocampus. *Neuron*, *36*(3), 527–538. https://doi.org/10.1016/S0896-6273(02)01001-2

Díaz-Mataix, L., Chacon Ruiz Martinez, R., Schafe, G. E., Ledoux, J. E., Doyère, V., & Author, C. B. (2013). Detection of a temporal error triggers reconsolidation of amygdala-dependent memories. *Current Biology*, *23*(6), 467–472. https://doi.org/10.1016/j.cub.2013.01.053

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. https://doi.org/10.1016/S0166-4328(98)00079-5

Dubue, J. D., McKinney, T. L., Treit, D., & Dickson, C. T. (2015). Intrahippocampal anisomycin impairs spatial performance on the Morris water maze. *Journal of Neuroscience*, *35*(31), 11118–11124. https://doi.org/10.1523/JNEUROSCI.1857-15.2015

Eisenberg, M., & Dudai, Y. (2004). Reconsolidation of fresh, remote, and extinguished fear memory in medaka: Old fears don't die. *European Journal of Neuroscience*, *20*(12), 3397–3403. https://doi.org/10.1111/j.1460-9568.2004.03818.x

Felder, C. C. (1995). Muscarinic acetylcholine receptors: Signal transduction through multiple effectors. *The FASEB Journal*, *9*(8), 619–625. https://doi.org/10.1096/fasebj.9.8.7768353

Giovannini, M. G., Rakovska, A., Benton, R. S., Pazzagli, M., Bianchi, L., & Pepeu, G. (2001). Effects of novelty and habituation on acetylcholine, GABA, and glutamate release from the frontal cortex and hippocampus of freely moving rats. *Neuroscience*, *106*(1), 43–53. https://doi.org/10.1016/S0306-4522(01)00266-4

Hasselmo, M. E. (2006). The role of acetylcholine in learning and memory. *Current Opinion in Neurobiology*, *16*(6), 710–715. https://doi.org/10.1016/j.conb.2006.09.002

Hasselmo, M. E., & Sarter, M. (2011). Modes and models of forebrain cholinergic neuromodulation of cognition. *Neuropsychopharmacology*, *36*(1), 52–73. https://doi.org/10.1038/npp.2010.104

Horowitz, L. F., Hirdes, W., Suh, B.-C., Hilgemann, D. W., Mackie, K., & Hille, B. (2005). Phospholipase C in living cells: Activation, inhibition, Ca 2 requirement, and regulation of M current. *The Journal of General Physiology*, *126*(3), 243–262. https://doi.org/10.1085/jgp.200509309

Jardine, K. H., Wideman, C. E., Macgregor, C., Sgarbossa, C., Orr, D., Mitchnick, K. A., & Winters, B. D. (2020). Activation of cortical M1 muscarinic receptors and related intracellular signaling is necessary for reactivation-induced object memory updating. *Scientific Reports*, *10*, 9209. https://doi.org/10.1038/s41598-020-65836-x

Jarome, T. J., Ferrara, N. C., Kwapis, J. L., & Helmstetter, F. J. (2015). Contextual information drives the reconsolidation-dependent updating of retrieved fear memories. *Neuropsychopharmacology*, *40*, 3044–3052. https://doi.org/10.1038/npp.2015.161

Jarome, T. J., & Helmstetter, F. J. (2013). The ubiquitin–proteasome system as a critical regulator of synaptic plasticity and long-term memory formation. *Neurobiology of Learning and Memory*, *105*, 107–116. https://doi.org/10.1016/j.nlm.2013.03.009

Jarome, T. J., Werner, C. T., Kwapis, J. L., & Helmstetter, F. J. (2011). Activity dependent protein degradation is critical for the formation and stability of fear memory in the amygdala. *PLoS One*, *6*(9), 24349. https://doi.org/10.1371/journal.pone.0024349

Klinkenberg, I., Sambeth, A., & Blokland, A. (2011). Acetylcholine and attention. *Behavioural Brain Research*, *221*, 430–442. https://doi.org/10.1016/j.bbr.2010.11.033

Krawczyk, M. C., Millan, J., Blake, M. G., & Boccia, M. M. (2021). Critical role of hippocampal muscarinic acetylcholine receptors on memory reconsolidation in mice. *Neurobiology of Learning and Memory*, *177*, 107360. https://doi.org/10.1016/j.nlm.2020.107360

Kwapis, J. L., Alaghband, Y., Keiser, A. A., Dong, T. N., Michael, C. M., Rhee, D., … Wood, M. A. (2019). Aging mice show impaired memory updating in the novel OUL updating paradigm. *Neuropsychopharmacology*, *45*, 337–346. https://doi.org/10.1038/s41386-019-0438-0

Lee, J. L. C. (2008). Memory reconsolidation mediates the strengthening of memories by additional learning. *Nature Neuroscience*, *11*(11), 1264–1266. https://doi.org/10.1038/nn.2205

Lee, J. L. C. (2009). Reconsolidation: Maintaining memory relevance. *Trends in Neurosciences*, *32*(8), 413–420. https://doi.org/10.1016/j.tins.2009.05.002

Lee, S.-H., Choi, J.-H., Le, N., Lee, H.-R., Kim, J.-I., Yu, N.-K., Choi, S. L., Lee, S. H., Kim, H., & Kaang, B.-K. (2008). Synaptic protein degradation underlies destabilization of retrieved fear memory. *Science*, *319*, 1253–1256. https://doi.org/10.1126/science.1153019

McGaugh, J. L. (2000). Memory: A century of consolidation. *Science*, *287*(5451), 248–251. https://doi.org/10.1126/science.287.5451.248

Morris, R. G. M., Inglis, J., Ainge, J. A., Olverman, H. J., Tulloch, J., Dudai, Y., & Kelly, P. A. T. (2006). Memory reconsolidation: Sensitivity of spatial memory to inhibition of protein synthesis in dorsal hippocampus during encoding and retrieval. *Neuron*, *50*, 479–489. https://doi.org/10.1016/j.neuron.2006.04.012

Murai, T., Okuda, S., Tanaka, T., & Ohta, H. (2007). Characteristics of object location memory in mice: Behavioral and pharmacological studies. *Physiology and Behavior*, *90*, 116–124. https://doi.org/10.1016/j.physbeh.2006.09.013

Nader, K., & Hardt, O. (2009). A single standard for memory: The case for reconsolidation. *Nature Reviews Neuroscience*, *10*, 224–234. https://doi.org/10.1038/nrn2590

Nader, K., Schafe, G. E., & Le Doux, J. E. (2000). Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. *Nature*, *406*(6797), 722–726. https://doi.org/10.1038/35021052

Ossenkopp, K.-P., Sutherland, C., & Ladowsky, R. L. (1986). Motor activity changes and conditioned taste aversions induced by administration of scopolamine in rats: Role of the area Postrema. *Pharmacology Biochemistry & Behavior*, *25*, 269–276. https://doi.org/10.1016/0091-3057(86)90265-0

Paxinos, G., & Watson, C. (2007). *The rat brain in stereotaxic coordinates*. 6th ed, San Diego: Elsevier Academic Press.

Sarter, M., & Bruno, J. P. (1997). Cognitive functions of cortical acetylcholine: Toward a unifying hypothesis. *Brain Research Reviews*, *23*, 28–46. https://doi.org/10.1016/S0165-0173(96)00009-4

Scavuzzo, C. J., LeBlancq, M. J., Nargang, F., Lemieux, H., Hamilton, T. J., & Dickson, C. T. (2019). The amnestic agent anisomycin disrupts intrinsic membrane properties of hippocampal neurons via a loss of cellular energetics. *Journal of Neurophysiology*, *122*(3), 1123–1135. https://doi.org/10.1152/jn.00370.2019

Sharma, A. V., Nargang, F. E., & Dickson, C. T. (2012). Neurosilence: Profound suppression of neural activity following intracerebral administration of the protein synthesis inhibitor anisomycin. *Journal of Neuroscience*, *32*(7), 2377–2387. https://doi.org/10.1523/JNEUROSCI.3543-11.2012

Stiver, M. L., Cloke, J. M., Nightingale, N., Rizos, J., Messer, W. S., & Winters, B. D. (2017). Linking muscarinic receptor activation to

UPS-mediated object memory destabilization: Implications for long-term memory modification and storage. *Neurobiology of Learning and Memory*, *145*(October), 151–164. https://doi.org/10.1016/j.nlm.2017.10.007

Stiver, M. L., Jacklin, D. L., Mitchnick, K. A., Vicic, N., Carlin, J., O'Hara, M., & Winters, B. D. (2015). Cholinergic manipulations bidirectionally regulate object memory destabilization. *Learning & Memory*, *22*(4), 203–214. https://doi.org/10.1101/lm.037713.114

Suzuki, A., Josselyn, S. A., Frankland, P. W., Masushige, S., Silva, A. J., & Kida, S. (2004). Memory reconsolidation and extinction have distinct temporal and biochemical signatures. *The Journal of Neuroscience*, *24*(20), 4787–4795. https://doi.org/10.1523/JNEUROSCI.5491-03.2004

Warburton, E. C., Koder, T., Cho, K., Massey, P. V., Duguid, G., Barker, G. R. I., Aggleton, J. P., Bashir, Z. I., & Brown, M. W. (2003). Cholinergic neurotransmission is essential for perirhinal cortical plasticity and recognition memory. *Neuron*, *38*(6), 987–996. https://doi.org/10.1016/s0896-6273(03)00358-1

Watanabe, H., & Shimizu, H. (1989). Effect of anticholinergic drugs on striatal acetylcholine release and motor activity in freely moving rats studied by brain microdialysis. *The Japanese Journal of Pharmacology*, *51*(1), 75–82. https://doi.org/10.1254/jjp.51.75

Wideman, C. E., Nguyen, J., Jeffries, S. D., & Winters, B. D. (2021). Fluctuating NMDA receptor subunit levels in perirhinal cortex relate to their dynamic roles in object memory destabilization and reconsolidation. *International Journal of Molecular Sciences*, *22*, 67. https://doi.org/10.3390/ijms22010067

Winters, B. D., & Bussey, T. J. (2005). Transient inactivation of perirhinal cortex disrupts encoding, retrieval, and consolidation of object recognition memory. *The Journal of Neuroscience*, *25*(1), 52–61. https://doi.org/10.1523/JNEUROSCI.3827-04.2005

Winters, B. D., Forwood, S. E., Cowell, R. A., Saksida, L. M., & Bussey, T. J. (2004). Double dissociation between the effects of peri-postrhinal cortex and hippocampal lesions on tests of object recognition and spatial memory: Heterogeneity of function within the temporal lobe. *The Journal of Neuroscience*, *24*(26), 5901–5908. https://doi.org/10.1523/JNEUROSCI.1346-04.2004

Winters, B. D., Saksida, L. M., & Bussey, T. J. (2006). Paradoxical facilitation of object recognition memory after infusion of scopolamine into perirhinal cortex: Implications for cholinergic system function. *The Journal of Neuroscience*, *26*(37), 9520–9529. https://doi.org/10.1523/JNEUROSCI.2319-06.2006

Winters, B. D., Tucci, M. C., & Dacosta-Furtado, M. (2009). Older and stronger object memories are selectively destabilized by reactivation in the presence of new information. *Learning and Memory*, *16*(9), 545–553. https://doi.org/10.1101/lm.1509909

Winters, B. D., Tucci, M. C., Jacklin, D. L., Reid, J. M., & Newsome, J. (2011). On the dynamic nature of the engram: Evidence for circuit-level reorganization of object memory traces following reactivation. *Journal of Neuroscience*, *31*(48), 17719–17728. https://doi.org/10.1523/JNEUROSCI.2968-11.2011

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Huff, A. E., McGraw, S. D., & Winters, B. D. (2022). Muscarinic (M_1) cholinergic receptor activation within the dorsal hippocampus promotes destabilization of strongly encoded object location memories. *Hippocampus*, *32*(1), 55–66. https://doi.org/10.1002/hipo.23396