Novelty enhances memory persistence and remediates propranolol-induced deficit via reconsolidation

Novelty enhances memory persistence and remediates propranolol-induced deficit via reconsolidation

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HIGHLIGHTS

- A novel event can reverse memory impairment caused by interfering reconsolidation with a noradrenergic β-blocker.
- Immediate-early gene, zif268, is not required for protein synthesis-dependent reconsolidation of appetitive spatial memory.
- A novel event can reverse the memory impairment caused by blocking reconsolidation with the noradrenergic beta-blocker propranolol.

ARTICLE INFO

ABSTRACT

Memory reactivation has been shown to open a time window for memory modulation. The majority of the methodological or pharmacological approaches target disruption of reconsolidation to weaken aversive memories. However, methods to improve appetitive memory persistence through reconsolidation or to reverse drug-induced reconsolidation impairment are limited. To improve memory persistence, previous studies show that a novel event, introduced around the time of memory encoding, enables the persistence of an otherwise decayed memory. This is mainly through a memory consolidation process. The current study first investigated if a novel event introduced during memory reactivation improves memory persistence through reconsolidation. Using a rodent appetitive spatial paradigm, similar to the human everyday experience of recalling where an item is located, a novel event around memory reactivation facilitated the persistence of spatial memory. This facilitation did not occur when the novel event was omitted and the protein synthesis-dependent reconsolidation was not affected by zif268 anti-sense in the dorsal hippocampus. Furthermore, beta-adrenergic antagonists, propranolol, impaired reconsolidation of appetitive spatial memory and contextual fear conditioning. A novel event after memory reactivation could reverse this impairment due to propranolol. Together, this study provides methods and confirmation for improving memory persistence during memory reactivation and reconsolidation.

1. Introduction

Developing methods and identifying mechanisms for improving memory persistence for the benefit of cognitive wellbeing are central themes in memory research. In a complex environment, the location of objects of interest, such as where one's car is parked or where food is placed, needs to be remembered for effective navigation or retrieval. However, encounters with these objects are often very brief, leading to short-lasting memories that fade away over time. Similar to memory decay over time in humans, time-dependent memory decay is also observed in a rodent behavioral paradigms that are used for understanding the neurobiology of memory persistence (Moncada et al., 2015; Wang and Morris, 2010). A key paradigm of this kind involves

training animals to remember the location of food in an open arena and then to use this spatial memory to effectively obtain more food later when facing multiple choices (Wang et al., 2010; Salvetti et al., 2014). This appetitive spatial paradigm in animals is highly comparable to our daily human experience and provides a good model for developing strategies for memory improvement.

Similar to memory decay that is observed at the behavioral level, neural plasticity decays, a key physiological observation closely associated with learning and memory, also decays at the synaptic level (Martin et al., 2000; Wang and Morris, 2010). For example, in the hippocampal slices, weak stimulation typically leads to long-term potentiation that decays to baseline after 2–4 h (Frey and Morris, 1997). Importantly, this type of decay can be prevented if strong stimulation in

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a second pathway that converges to an overlapping set of neurons or synapses is applied around the time of weak stimulation. This phenomenon has been extensively observed and it provides the foundation for the synaptic tagging and capture theory (Frey and Morris, 1997; Redondo and Morris, 2011; Shivarama Shetty and Sajikumar, 2017).

The principle that a strong event can facilitate the persistence of a weak memory has also been demonstrated behaviorally. The process of facilitating memory persistence using a second behavioral event that provides the required protein synthesis has been called behavioral tagging (Moncada et al., 2015; Redondo and Morris, 2011; Vishnoi et al., 2016; Wang and Morris, 2010). For example, it has been shown in inhibitory avoidance, a short-term memory can last for a long time when exploration in a novel open field occurs shortly before or after the avoidance learning (Moncada and Viola, 2007). Similarly, place memory of where food reward is hidden that typically fades away over 24 h can remain longer if exploration in a novel box occurs before or after encoding of the place (Wang et al., 2010). The time window during which novelty can boost memory persistence in an inhibitory avoidance task ranges from 1 h before to 30 min after encoding (Moncada and Viola, 2007). It is hypothesized that novelty triggers plasticity-related proteins that can be captured by encoding-activated synapses and lead to long-term change, hence called behavioral tagging (Moncada and Viola, 2007; Redondo and Morris, 2011). This principle has been shown to enable persistence of a wide range of memory types, such as contextual fear memory, conditioned taste memory, and object recognition memory (Ballarini et al., 2009). Besides novelty, other types of tasks, such as reward learning in a T-maze, can also facilitate the persistence of spatial memory that would otherwise fade (Salvetti et al., 2014).

While facilitating memory persistence during encoding is a robust phenomenon, it is yet to be determined whether the same principle of facilitating memory persistence using novel events can be recapitulated at the time of memory reactivation after memory encoding is finished. The answer to this question may allow a substantial extension of the time window beyond the initial encoding for improving memory persistence. Memory reactivation has been shown to engage molecular mechanisms to enable long-lasting memory in a process called reconsolidation (Tronson and Taylor, 2007). Blocking protein synthesis (Nader et al., 2000), noradrenergic receptors (Debiec and Ledoux, 2004) and other neuronal signaling pathways (Barak et al., 2013) after memory reactivation can effectively impair subsequent memory recall. Together, these studies provide methods to weaken negative memories, such as cues, that are associated with footshocks or other aversive consequences. However, strategies to improve persistence of appetitive memory through reactivation and reconsolidation are limited. Hence, a key objective of this study was to determine whether it is possible to provide evidence to 'gain function' in making appetitive memories last using novelty at the time of memory reactivation, which will lead to a new translatable method to improve cognitive function.

This study first investigated whether behavioral tagging and capture occurs at the time of memory reactivation and reconsolidation to facilitate subsequent memory persistence. To this end, four sets of experiments using two behavioral paradigms and two pharmacological approaches were conducted. In Experiment 1, I examined if novelty introduced during memory reactivation could sufficiently facilitate the persistence of spatial memory in an appetitive paradigm. In Experiment 2, I examined if knocking down immediate-early gene zif268, that has been previously shown to selectively impair reconsolidation of fear memory (Lee et al., 2004; Trent et al., 2015), could also interfere with reconsolidation of appetitive spatial memory. An additional experiment 4 was designed to add control tests for studies in experiments 1 and 2. In Experiment 3, I examined if novelty introduced after memory reactivation could reverse memory reconsolidation impairment caused by beta noradrenergic antagonist (Debiec and Ledoux, 2004) in contextual fear conditioning.

2. Materials and methods

2.1. Animals

Adult male Lister-hooded rats (12–14 weeks old, Charles River, UK) were used in the first two experiments (n = 16 each) that involved a spatial appetitive task. They have better color vision than other commonly used albino strains. This was also to be consistent with our previous studies (Wang et al., 2010; Salvetti et al., 2014). Adult male Sprague-Dawley rats (12–14 weeks old, n = 32 for 4 groups at n = 8 per group) were used in Experiment 3 to be consistent with previous fear conditioning and reconsolidation studies (Wang et al., 2009). There were group housed with 4 rats per cage on a 12 h light/dark cycle. Experimental procedures were performed during the light cycle. They were acclimatized to the animal room for 3 days or more and handled for 3–5 days during which they had unlimited access to food and water. During training of the appetitive spatial task, rats in Experiments 1 and 2 had unlimited access to water while limited amount of regular rodent chow (18–25 g per rat, given at about 1 h after the behavioral session) was provided daily to maintain them at 90–95% of free-feeding weight. The light food restriction was used to increase the motivation for food searching in the appetitive spatial task. An additional group of 16 male Lister-hooded rats (Experiment 4) was handled and housed similarly for characterization of time-dependent memory decay in Fig. 1E and control studies shown in Fig. 5D and F. Rats in Experiment 3 had unlimited access to water and food throughout the experiment. All procedures were approved by local veterinary scientific officers, conducted by Home Office license holders, and adhered to the UK Home Office regulations of animal experimentation (Animals (Scientific Procedures) Act 1986).

2.2. Apparatus

Event arena. An open-top square arena (Fig. 1A) was made of clear Plexiglas walls and white Plexiglas floor (160 cm × 160 cm x 40 cm, in L x W x H) that was covered with sawdust (about 2 cm thick). A 10 cm gap located at the center of each wall provided a passageway for connecting to the start boxes (30 cm × 25 cm x 30 cm). The floor contained 7 × 7 convertible holes (6.5 cm in diameter, 20 cm apart) that could be used to place small Plexiglas wells that contained sand (i.e. sandwells). These Plexiglas wells were designed with a divider at the bottom to store inaccessible food pellets and were filled with sand mixed with ground food powder (5%). These two features were designed to provide similar odor cues across multiple sandwells. A red pyramid and a grey cube (about 10 cm × 10 cm x 40 cm) were located at columns 2 and 6 at row 4 to serve as landmarks. Various 2 dimensional and 3 dimensional visual cues were also provided on the walls of the room (Fig. 1A, Wang et al., 2012a). Chocolate-flavored supreme mini pellets (0.5 g per pellet, Bio-Serv, US) were used as food rewards. Further details can also be seen in our previous publication (Wang et al., 2010; Salvetti et al., 2014).

Exploration box. An open-top square box (100 cm × 100 cm x 45 cm) was made of clear Plexiglas walls and wooden floor with waterproof coating. Novel substrates, including plastic straws, pebbles, shredded papers, metal mesh wires, were placed on the floor to introduce novelty.

Conditioning chambers. Fear conditioning was done in chambers composed of 2 clear Plexiglas walls, 2 aluminum side panels, and 1 aluminum panel as ceiling (30 cm × 26 cm x 33 cm, Coulbourn Instruments, US). The floor was made of 18 stainless steel bars (0.5 cm in diameter, about 1 cm apart). The conditioning chambers were cleaned with disinfectant wipe (Virkon, UK) and dried with tissues between animals. A different chamber was used at the end of the conditioning and memory testing to examine context discrimination. This second box was composed of a curved wickered wooden panel that covered 3 walls and an opaque Plexiglas front door. The floor was lined
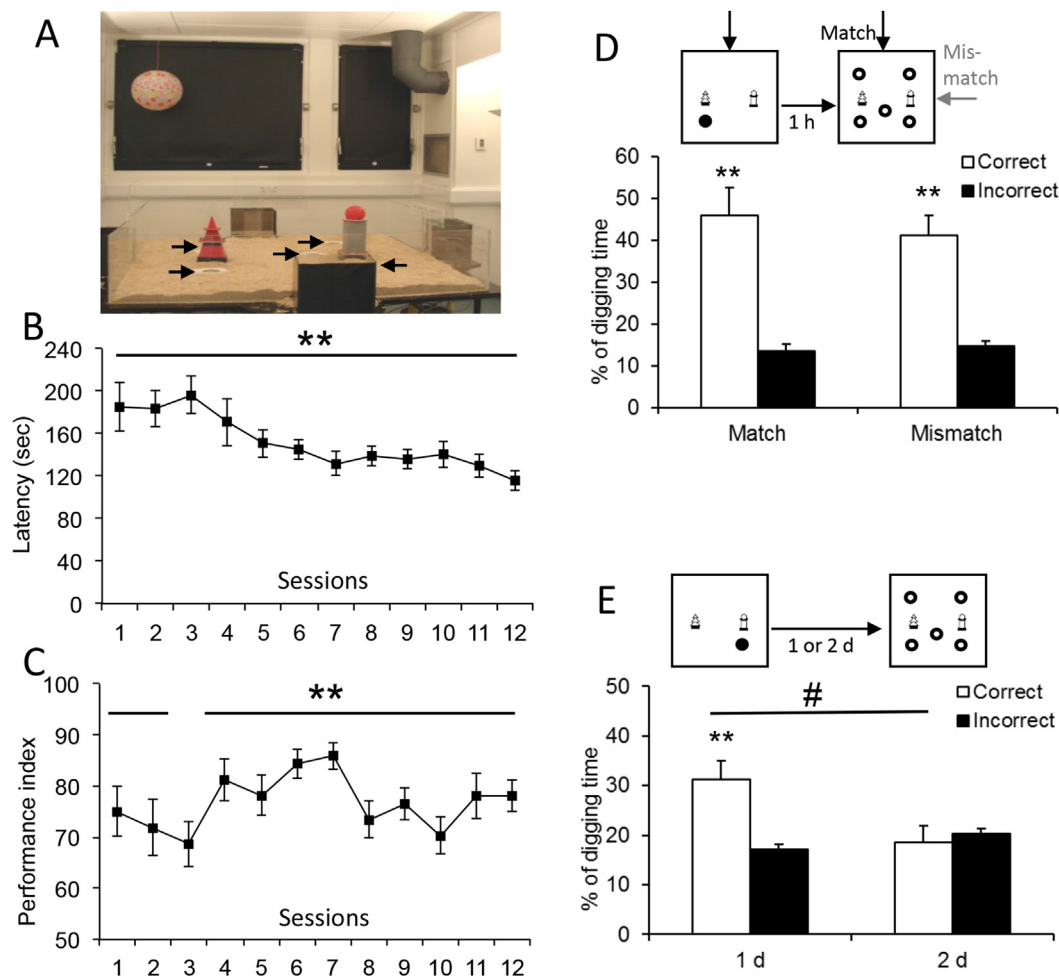
Fig. 1. Acquisition of the appetitive spatial memory task. (A) A picture of the event arena and the experimental room for studying spatial appetitive memory. Extra-maze cues and two intra-maze landmarks were visible and arrows indicated 5 representative sandwell locations. (B) The latency for rats to retrieve food pellets was significantly reduced over the 12 training sessions. The linear trend was significant (**$p < 0.001$). (C) The performance index was significantly above chance (which was 50) during the training (** all $p \leq 0.01$, except session no. 3). (D) Behavioral procedures shown on top indicate a probe test with 5 non-reward sandwells (open circles) given 1 h after encoding with either matched or mismatched start locations. Percentages of time digging at the correct location and at incorrect location (averaged) were measured during the probe test. The percentage of time digging at the correct location was significantly above chance (which was 20%) in both conditions (**$p < 0.005$). No significant difference between conditions was found. (E) Behavioral procedures shown on top indicate a probe test given 1 day or 2 days after encoding. The percentage of time digging at the correct location was significantly above chance (which was 20%) 1 day, but not 2 days later (**$p < 0.01$, day effect #$p = 0.05$). Data = mean ± standard error of the mean (s.e.m.); n = 16.

with fluffy wood shreds in the plastic tray that was lightly scented (natural lemon flavoring, 10% in water, 0.2 mL spread on the tray under the wood shreds). The wood shreds was renewed and the tray and chamber was cleaned with diluted alcohol, water, and dried with towels across animals.

2.3. Stereotaxic surgery

Rats (body weight 305–344 g) in experiment 2 received bilateral cannulation targeting the dorsal hippocampus prior to behavior training. They were anesthetized with isoflurane and mounted on a standard stereotaxic frame (Kopf Instruments, US). Analgesics (Rimadyl, Pfizer, UK) was injected at the beginning of surgery and provided in drinking water for 3 days after the surgery. Guide cannulas, 26 gauge stainless steel (Plastics One, US), were implanted using the following coordinates: 4 mm posterior to and ± 3.0 mm lateral from bregma and 3.0 mm below dura (Paxinos and Watson, 2004). To prevent blockade, dummy cannula with caps were kept in the guide cannula and were removed temporarily for mock and drug infusions. Rats recovered from surgery in a week by showing normal body weight gain. Training day 1 started at about 2 weeks after surgery. At the end of the

experiment, the brains were extracted, post-fixed in formalin, cryoprotected in sucrose solution, and sliced with cryostat (30 μm thick) for visualizing the location of cannula tips. To acclimatize the animals with infusion, they were handled with dummy cannulas removed and replaced again. Rats in experiment 4 received 12 days of training, 5 probe tests (Figs. 1E and 5F), with interleaving training sessions. They underwent dorsal hippocampus cannulation and had 7–10 days of recovery. They received retraining and 2 probe tests (Fig. 5D).

2.4. Drugs and infusions

Anisomycin (Sigma-Aldrich) was dissolved in 1 N HCl, diluted with sterile physiological saline, and adjusted to pH 7.4 with 1 N NaOH to reach the final concentration of 125 μg/μL. Bilateral 1 μL was infused via injection cannula (33 gauge, 0.5 mm below the guide), PE tubes, and microsyringes (5 μL, SGE). The pump was set to deliver steady infusion at 0.25 μL/min/side for 4 min. The injection cannulas remained in the guide cannulas for one additional minute after the infusion, after which the dummy cannulas were placed. Based on previous studies showing a selective role of zif268 in memory reconsolidation (Lee et al., 2004, 2005; Théberge et al., 2010), oligodeoxynucleotides of zif268

antisense (5′-GGT-AGT-TGT-CCA-TGG-TGG-3′) and mis-sense (5′-GTG-TTC-GGT-AGG-GTG-TCA-3′) were produced from the same supplier (Alta Bioscience, UK) and prepared at the same dose as these previous published studies. Both were resuspended in sterile PBS (pH 7.4) to yield a concentration of 2 μmol/μL. A volume of 1 μL was infused per hemisphere at 90 min before memory reactivation over 8 min of duration. Rats underwent acclimatization with the dummy cannula removed/replaced and handling while no infusion was applied. No obvious stressful signs or struggling were observed on the days of drug infusion. In experiment 3 and in experiment 4 (Fig. 5F), propranolol (Sigma-Aldrich) was dissolved in sterile physiological saline (10 mg/mL) and delivered by intraperitoneal injection (1 mL/kg).

2.5. The spatial appetitive task in the event arena

Pre-training. Rats were given chocolate pellets in sandwells in their home cages for 30 min per day for 3 days to familiarize with the food reward. They were exposed to the event arena at one quadrant a time with one sandwell containing food pellets at the farthest corner. After experiencing all 4 quadrants, they were exposed to the left or right half of the arena with one sandwell containing food pellets each time in two sessions. Next, they were exposed to the entire arena with one sandwell containing food pellets in the center of the arena. Finally, they received 2 sessions of pre-training: a trial with a rewarded sandwell, followed by 40 min later a trial with 1 rewarded sandwell at the same location and 2 other non-rewarded ones. After these sessions, they were familiarized with the procedure aspects of the study, including digging the sandwell, finding the food reward, and carrying the reward to the start box where they normally ate.

Training. Rats received an encoding trial with one sandwell containing food pellets presented in the event arena. About 40 min later, they received a retrieval choice trial with 1 rewarded sandwell that matched to the encoding location and 4 non-rewarded sandwells at different locations. Each trial began with placing the rat in the start box that contained a pellet to encourage the rats to eat in the start box and a small pot of water. About 1 min after placing the rat to allow for consumption of the pellet, the door would open, remotely controlled by a computer program, and the rat entered the arena, searched the sandwell, and dug for the pellets. They collected the first pellet and returned to the start box to eat. After eating the first pellet, they typically returned to the sandwell, found the next pellet, and ate at the start box again. The trial stopped after they collected 3 pellets at the encoding trial and 3 pellets at the retrieval trial. The rewarded location changed across animals within every training day so that the rat could not locate the rewarded sandwell based on the previous rat's path. For each rat, the rewarded location was changed from day to day in a counterbalanced matter so they experienced rewarded spots that were near or far, and at left or right, in relation to the start box on different days. The start box changed across days so the rats experienced all four possible start locations.

Probe tests. After 12 sessions of training with encoding and retrieval paired trials, they underwent probe tests in various conditions. First, a non-rewarded sandwell was presented as a reactivation trial at 24 h after an encoding trial with 3 pellets. The rats were then exposed (or not) to a novel box for 5 min after the reactivation trial. They were tested at 24 h after reactivation with 5 non-rewarded sandwells for 60 s (i.e. a probe test). The time they spent on digging each sandwell was recorded and used for calculating the correct and incorrect digging percentage. Second, a 1-pellet rewarded sandwell was presented as a reactivation trial at 24 h after an encoding trial with 3 pellets at a matching location or non-matching location. The rats were then exposed to a novel box for 5 min after the reactivation trial. They received a probe test at 24 h after reactivation. Third, a non-rewarded sandwell was presented at an encoding trial, followed by 5 min novel box exposure at 30 min later. They received a probe test at 24 h after encoding. Further conditions were described below in the results. To

avoid extinction of digging behavior due to probe tests, a regular training session with encoding and retrieval paired trials were introduced in between probe tests. In experiment 2, rats received an encoding trial with 3 pellets on one day and a reactivation trial with 3 pellets at 24 h later. A protein synthesis inhibitor, anisomycin (n = 8, randomly assigned), or vehicle (n = 8) was infused in the dorsal hippocampus immediately after reactivation. They had a probe test on the following day. After regular training, the role of zif268 in reconsolidation was examined. Rats underwent similar encoding, reactivation, and probe tests. The only difference was the timing of antisense or missense (n = 16, order of infusion counterbalanced across animals) that was infused at 90 min before reactivation (Théberge et al., 2010). In experiment 4 (Fig. 5D), animals received anisomycin or vehicle infusion without reactivation at 24 h after encoding and 24 h before a probe test.

2.6. Contextual fear conditioning

Training. Rats were familiarized with handling and cage transportation for 5 days prior to the training. They were put in the conditioning chamber for 240 s. Two brief mild footshocks (0.5 mA, 1 s, scrambled), were delivered through the grid floor at 119 s and 179 s. They were immediately returned to the home cage at the end of the session. One day later, they were returned to the same chamber for 90 s for memory reactivation. After this, they were randomly divided into 4 groups: Groups 1 and 2 received saline injection, groups 3 and 4 received propranolol injection. While groups 1 and 3 remained in the home cages after injection, groups 2 and 4 received 5 min box exploration at 30 min after injection.

Testing. Rats were placed in the conditioning chamber for a 90 s post-reactivation short-term memory test at 4 h after reactivation. The short reactivation and memory test was carefully chosen to prevent extinction (Mamiya et al., 2009). One day and seven days after reactivation, they were place in the conditioning chamber again for 120 s for assessing their post-reactivation long-term memory. Three hours after the last test, they were placed in a second distinct context for 120 s for measuring the generalization of freezing to a non-conditioned environment. No footshock was delivered during testing.

2.7. Behavior measurement, data collection, statistical analysis

All behavior described in results was measured by the experimenter 'blind' to the condition or the drug treatment that the animal received. The condition or group identity was revealed after individual behavioral measurement was done in order to proceed with subsequent statistical analysis. The training performance in the event arena was shown by two measurement: First, the latency at obtaining all three pellets in the retrieval trial was to show how efficient the animal was at the performing the task. Second, the performance index at obtaining the first reward at the retrieval trial was used to show how accurate the animal was at retrieving the spatial information after a post-encoding delay. It was calculated by 100 – no. of errors * 25. By chance, they could make 2 errors to find the reward randomly and led to performance index = 50. During probe tests, the correct digging performance was measured by 'correct digging time/total digging time * 100%'. The incorrect digging average was measured by 'digging time in 4 incorrect locations/total digging time * 100%/4'. The chance level for the probe test is 100%/5 sandwells = 20%. Paired 2-tailed t-tests were used to compare correct digging percentage in paired conditions. One-sample t-tests were used to examine if correct digging percentage was significantly different from chance (20%) for each condition. Although the prediction was one directional (i.e. higher than chance), more stringent 2-tailed tests were applied. In one condition in Experiment 1 when 3 measurements on the percentage of time at encoding, reactivated, and incorrect locations were compared, one-way repeated-measure ANOVA was applied. Type one error, alpha, was set at 0.05. In the fear

conditioning experiment, the percentage of time that the rats remained immobilized (except for breathing, Blanchard and Blanchard, 1969), was measured and formed the index of freezing. Two-way AVOVAs (Veh/Prop, without Box/with Box) were used for group comparison. To further validate the source of effect, post hoc tests were done with Bonferroni correction that set the type one error at a more stringent 0.0167.

3. Results

3.1. Novelty facilitates memory persistence through behavioral tagging during memory reactivation and reconsolidation

In Experiment 1, a highly significant linear decline of latency to retrieving all rewards was seen across 12 training sessions (Fig. 1B, $F_{1,15} = 20.81$, $p < 0.0001$). This suggests that rats gradually learned to obtain the rewards efficiently in the retrieval trial. Their performance index was significantly above chance (50%) across most of the training sessions (Fig. 1C, one-sample t tests, $t_{15} = 2.91–8.885$, all $p = 0.01–0.0001$, except for session 3, $t_{15} = 1.86$, $p = 0.083$). This suggests that they maintained the information from the encoding trial and chose the correct, rewarded sandwell among 4 non-rewarded others highly accurately during the retrieval trial. After these training sessions, several conditions were introduced to investigate the memory persistence at probe tests after various types of encoding, delay, or reactivation. For example, when a probe test was done at 1 h after encoding, rats showed good memory, indicated by longer digging at the correct location (Fig. 1D, $t_{15} = 3.75$, $p = 0.002$). The memory remained good even when the start location between encoding and probe test were mismatched (e.g. encode from the north and test from the east, Fig. 1D). Percentage of time digging at the correct location was significantly above chance in the mismatched condition ($t_{15} = 4.39$, $p = 0.001$). There was no significant difference between these two conditions ($t_{15} = 0.52$, $p = 0.61$). This suggests that rats likely used allocentric cues, rather than solely relied on an egocentric strategy, to perform in this spatial task. When animals received 3 pellets of reward during encoding (experiment 4), digging at the correct sandwell was significantly above chance 24 h later ($t_{15} = 3.11$, $p = 0.007$) but not 48 h later ($t_{15} = -0.44$, $p = 0.67$). The difference between these two time delays was marginally significant ($t_{15} = 2.12$, $p = 0.051$).

Experiment 1 further examined if novelty introduced after memory reactivation enables spatial memory to last longer through memory reconsolidation. To achieve this, a 3-day protocol was introduced: an encoding trial first, a reactivation trial 24 h later, and a probe trial after another 24 h. In the first set of conditions, rats received an encoding trial with 3 pellets, a reactivation trial with a non-rewarded sandwell at the matching location. Thirty minutes after the reactivation, they were placed in a novel box (or this step omitted as control) for 5 min. On the probe test the following day, novelty, compared to control, led to a significant increase in digging percentage at the correct sandwell (Fig. 2A, $t_{15} = 3.35$, $p = 0.004$). Correct digging percentage was also highly significantly higher than chance in the novelty condition ($t_{15} = 4.51$, $p < 0.0001$). Together, these data suggest that novelty introduced after memory reactivation can improve subsequent memory persistence for 24 h.

To ensure that this improved memory persistence indeed occurs through reactivation and reconsolidation, the possibility that novelty improves memory through consolidation of the non-rewarded trial needed to be ruled out. To do this, conditions with no prior rewarded encoding were introduced. Rats received a non-rewarded trial in which they explored the arena, dug in the sandwell, and voluntarily returned to the start box after which the door was closed and trial stopped. Next, rats were or were not exposed to a novel box 30 min later. During the probe test the next day, digging performance was indifferent between conditions (Fig. 2B, $t_{15} = 0.5$, $p = 0.63$) and neither were significantly better than chance ($t_{15} = 0.11$, $p = 0.92$ for the no-box condition;
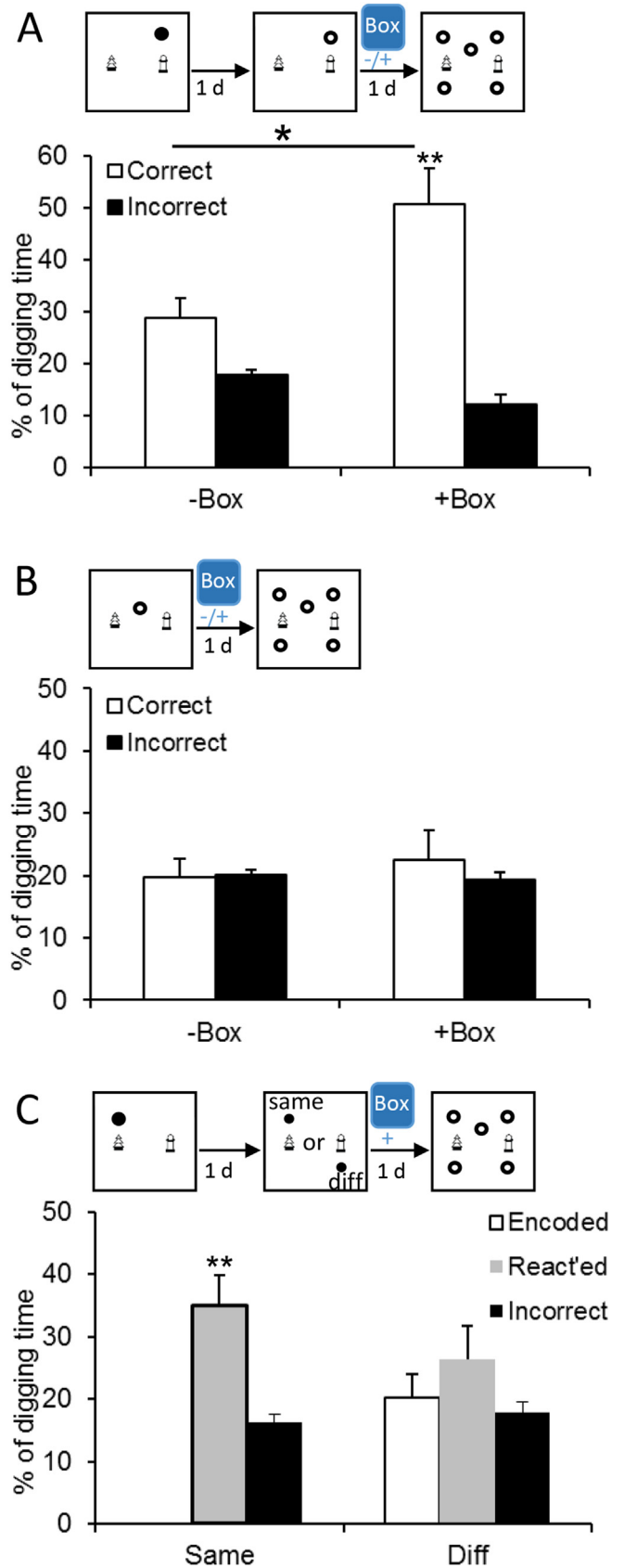
(caption on next page)

Fig. 2. Exploration in a novel box facilitated persistence of spatial appetitive memory through reconsolidation. (A) Behavioral procedures on top show rewarded encoding (a filled circle) in the event arena. One day (1 d) later, non-rewarded reactivation (an open circle) with or without exploration in a novel box (rounded square) was introduced. Another day later, a probe test with 5 non-rewarded sandwells was presented. Rats showed significantly higher correct digging percentage when they explored a novel box after reactivation than when they were not exposed to a novel box (*p < 0.005). The correct digging percentage in the novel box condition was significantly above chance (**p < 0.001). (B) Behavioral procedures on top show non-rewarded encoding (an open circle), with or without novel box, and a probe test the next day. The correct digging percentage in these conditions were not significantly different from each other and not above chance in either condition. (C) Behavioral procedures on top show rewarded encoding (a filled circle), weak rewarded reactivation with 1 pellet at a matching (same) or a nonmatching (different, diff) location followed by exploration in a novel box the next day, and a probe test after another one-day delay. Rats showed significantly higher percentage of digging at the encoded and reactivated location than chance in the matching condition (*p < 0.01). No significant difference among encoded, reactivated and other locations was found in the non-matching condition. Data = mean ± standard error of the mean (s.e.m.); n = 16.

$t_{15} = 0.55$, $p = 0.59$ for the novel-box condition). In our previous work, spatial novelty boosted the memory of a non-rewarded encoding trial (Salvetti et al., 2014). While the effect size was not large in that study, the variation was small and the memory was significantly above chance ($t_{15} = 2.38$, $p = 0.031$). When these two studies are compared, there is no significant group difference ($t_{30} = 1.12$, $p = 0.273$), which implies that this may be a borderline effect. When comparing the trial duration of non-rewarded encoding in both studies, animals in the current study voluntarily spent less time in the arena than the previous study ($t_{30} = -5.11$, $p < 0.001$), which may have contributed to shorter encoding duration and chance-level memory in Fig. 2B.

To further support the importance of reactivating a previous encoded memory that engages reconsolidation mechanisms, a rewarded sandwell was placed at a location that was matching to or non-matching to the encoded location. The prediction was that if the memory persistence seen in Fig. 2A was due to reconsolidation, rats in the matching condition would recall the previous memory and would show memory improvement after novelty; conversely, rats in the non-matching condition would not recall the previous location appropriately and not show persistence memory of that location after novelty. On the contrary, if the memory persistence in Fig. 2A was purely due to consolidation only and not reconsolidation, rats in the non-matching condition would search significantly longer in the new location that was different from encoded location on the previous day (Wang et al., 2010; Salvetti et al., 2014). Results (Fig. 2C) showed that the percentage of correct digging time was significantly above chance in the matching (same) condition ($t_{15} = 3.06$, $p = 0.008$). In the non-matching (different) condition, the percentage of time spent in digging the reactivated sandwell was not significantly higher than the time spent in digging the encoded or other sandwells ($F_{2,30} = 0.94$, $p = 0.4$).

To examine whether the sequence of reactivation and novelty is critical, our study using previously trained rats (Salvetti et al., 2014) showed that novelty either 1 h before or 30 min after reactivation facilitated memory persistence (Fig. 3A). When reactivation preceded the novel box, the correct digging percentage was higher than the no-box condition ($t_{15} = 2.56$, $p = 0.022$) and also above chance ($t_{15} = 3.45$, $p = 0.004$). When the novel box was introduced before reactivation, the correct digging percentage was higher than the no-box condition and also above chance ($t_{15} = 4.26$, $p = 0.001$).

To identify whether a shorter window of memory reactivation before the memory returns to baseline is sufficient for novelty to improve reconsolidation, a 2-day protocol was used: weak encoding (rewarded with 1 pellet), non-rewarded reactivation 6 h later, and probe test the next day. The rationale was twofold. First, this delay between encoding and reactivation was chosen because the weak memory does not
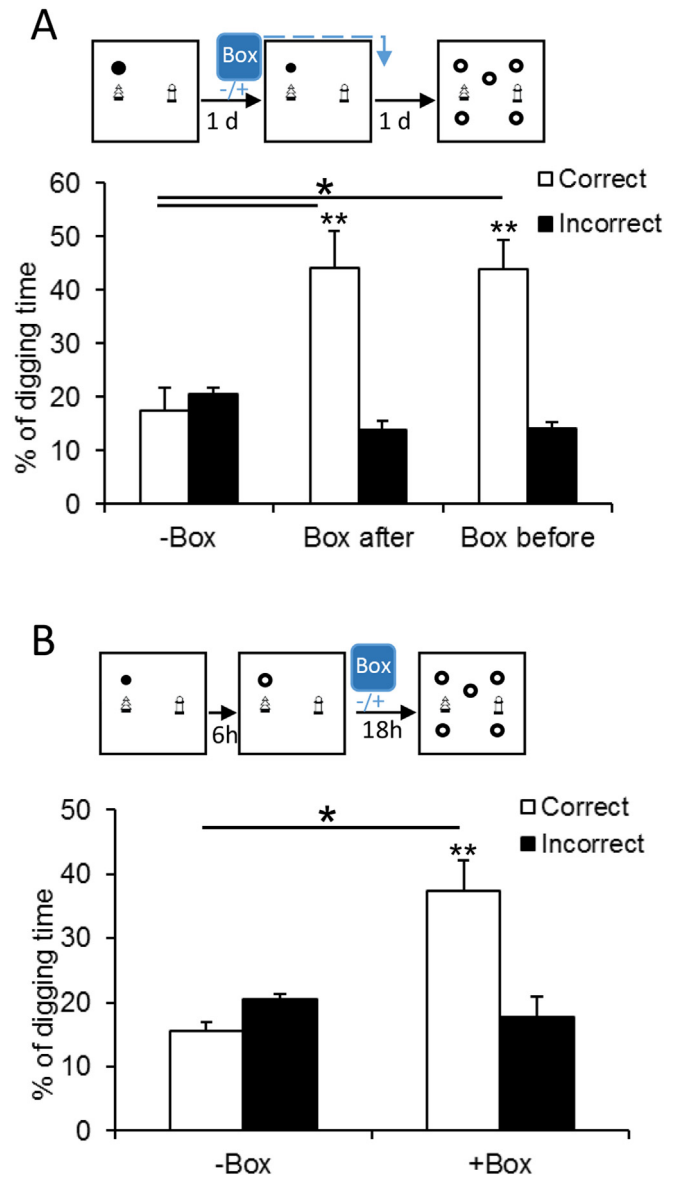
Fig. 3. Exploration in a novel box before or after memory reactivation facilitated memory persistence. (A) Behavioral procedures on top showed rewarded encoding (a filled circle) and weak rewarded (1 pellet) reactivation in the event arena followed by a probe test with 5 non-rewarded sandwells (open circles). A novel box (rounded square), introduced before or after memory reactivation, significantly facilitated memory persistence as shown by higher percentage of digging at the correct location (*p < 0.05, compared to the no-box condition) that was significantly above chance (**p < 0.005, compared to 20%). (B) Behavioral procedures on top show non-rewarded memory reactivation (an open circle) at 6 h after 1-pellet encoding (a filled circle) and a probe test at 24 h after encoding. The difference in correct digging percentage between conditions was significant (*P < 0.01) and the correct digging percentage was only significantly above chance in the novel box condition (**p < 0.005, compared to 20%). Data = mean ± standard error of the mean (s.e.m.); n = 16.

completely fade away after 6 h. In our previous consolidation study, we probed the animal after 1-pellet encoding and found partial memory at 6 h (correct digging at 35 ± 8%, 2-tailed test p = 0.09, 1-tailed test p = 0.045, when compared to chance, Wang, Redondo, Morris, unpublished). Second, time-dependent memory consolidation is likely to complete at this stage (McGaugh, 1966, 2000). For example, anisomycin given 3 h after training no longer blocks memory consolidation (Fulton et al., 2005; Robinson and Franklin, 2007). When given at 6 h,

instead of immediately, after memory reactivation, long-term memory is also not impaired (Nader et al., 2000).

Exploration in a novel box 30 min after reactivation also facilitated memory persistence, indicated by higher correct digging percentage than the no-box condition (Fig. 3B, t_{15} = 3.20, p = 0.006). The percentage of correct digging location was also above chance when the novel box was introduced (t_{15} = 3.56, p = 0.003). Together, data from Figs. 2 and 3 suggests that novelty can facilitate memory persistence of an appetitive spatial task through memory reactivation and reconsolidation.

3.2. Protein synthesis inhibitors, not zif268 anti-sense, impair memory persistence

Results from experiment 1 support the feasibility of improving memory persistence through memory reactivation and reconsolidation using novelty. Experiment 2 asked if memory impairment after interfering with reconsolidation could be reversed using novelty. Specifically, it aimed to examine if zif268 anti-sense selectively impairs memory reconsolidation. If this was the case, then this model could be used to further investigate whether novelty reverses the reconsolidation impairment. Rats underwent bilateral dorsal hippocampus cannulation (Fig. 4), post-surgery recovery, and 12 sessions of training.

In Experiment 2, rats also gradually learned to obtain rewards efficiently in the retrieval trial during 12 sessions of training. There was a highly significant linear decline of latency across sessions (Fig. 5A,
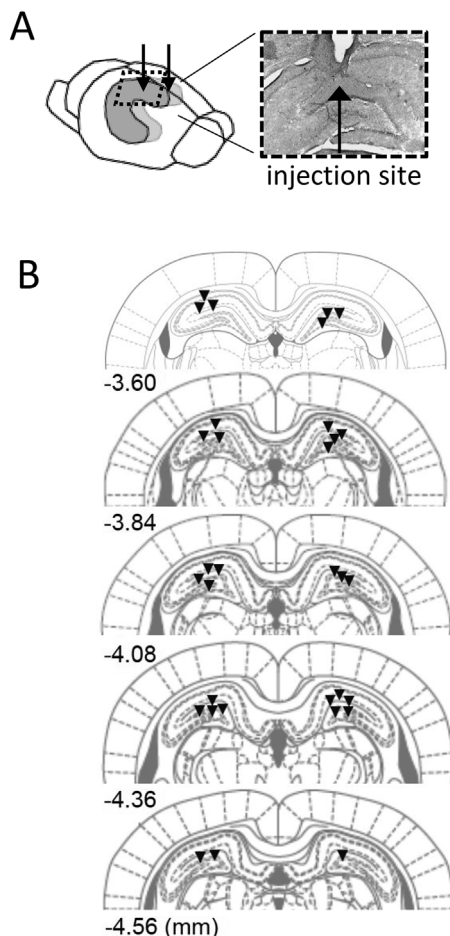
Fig. 4. Location of the cannula tips in the dorsal hippocampi. (A) A drawing of the rat brain with the hippocampus highlighted and a section showing cannula placement targeting the dorsal hippocampus. (B) Filled triangles indicate the location of cannula tips (i.e. infusion sites) in bilateral dorsal hippocampi from around 3.60 mm–4.56 mm posterior to the bregma.

$F_{1,15}$ = 12.70, p < 0.003). Their performance index was also significantly above chance at session 3 and the last 8 sessions of training (Fig. 5B, t_{15} = 2.42–6.26, p values = 0.03–0.001). Compared to Experiment 1, performance index was lower in the early training sessions in this experiment. This difference was likely due to the cannulation surgery that these rats received.

Previously studies showed that fear memory undergoes protein synthesis-dependent reconsolidation in the hippocampus and/or amygdala (Mamiya et al., 2009; Wang et al., 2009). Hence, this experiment first examined if protein synthesis in the hippocampus is required for memory reconsolidation in this appetitive paradigm. Rats received a 3-day protocol of an encoding trial with 3 pellets, a memory reactivation trial with 3 pellets 24 h later, immediately followed by anisomycin or vehicle infusion, and a probe test after a 24 h delay. More rewards at memory reactivation were used than in Experiment 1, to ensure lasting memory for studying memory disruption by drug intervention. Anisomycin, compared to vehicle infusions led to a lower percentage of digging time in the correct sandwell (Fig. 5C, t_{14} = 2.24, p = 0.042). The correct location digging percentage was significantly above chance in the vehicle treatment (p = 0.005), but not in the anisomycin treatment (p = 0.317). When the reactivation session was omitted before vehicle or anisomycin infusions in the hippocampus, the correct digging percentage was comparable in both conditions in experiment 4 (Fig. 5D, t_{15} = 0.15, p = 0.89).

While anisomycin given after reactivation impaired memory persistence, this does not allow us to dissociate the underlying processes of memory reconsolidation and memory consolidation. Specifically, it has been shown that both processes require protein synthesis (Nader et al., 2000; review in Dudai, 2012). The next probe tests targeted zif268 that was previously shown to selectively affect reconsolidation, but not consolidation (Lee et al., 2004, Lee et al., 2005; Théberge et al., 2010; Trent et al., 2015). Rats received the same 3-day protocol, but infusion of anti-sense, or mis-sense of zif268 was infused into hippocampus before memory reactivation and reconsolidation. No significant difference in the percentage of digging at correct sandwell between the two treatment conditions was found (Fig. 5E, t_{15} < 0.01, p = 0.97). The correct digging percentage in both treatments was significantly above chance (t_{15} = 3.54, p = 0.003 for mis-sense; t_{15} = 3.57, p = 0.003 for anti-sense). This suggests that zif268 antisense did not impair memory reconsolidation in this paradigm. Therefore, it was not feasible to answer whether novelty can selectively reverse impairment of memory reconsolidation using this drug target.

To determine whether the consolidation/reconsolidation impairment can be reversed using novelty, one proposal may be to introduce novelty following memory reactivation and anisomycin injection. However, the effect of novelty in facilitating memory persistence has been hypothesized to involve the production of plasticity-related proteins (Moncada et al., 2015; Redondo and Morris, 2011; Morris, 2006). Thus, anisomycin would also block proteins synthesis during novelty. An alternative drug candidate is proposed for investigating this. Noradrenergic receptor blocker, propranolol, has been shown to affect reconsolidation (supporting studies see below) without affecting novelty-facilitated memory persistence (Takeuchi et al., 2016). Therefore, in experiment 4, animals received saline (vehicle) injection after reactivation, propranolol injection after reactivation, and propranolol injection after reactivation followed by 5 min exploration in a novel box (with order counterbalanced across animals, and training interleaved between these tests). Percentage of correct digging, and therefore memory reconsolidation, was significantly lower following propranolol compared to vehicle (Fig. 5F, t_{15} = 2.25, p = 0.04). Animals exposed to a novel box after reactivation and propranolol injection had significantly better memory than those not exposed to a novel box (t_{15} = 3.39, p = 0.004). This suggests that reconsolidation impairments can be reversed which is further demonstrated below.

3.3. Novelty reverses reconsolidation impairment of contextual fear memory caused by beta-adrenergic antagonists

Experiment 3 examined whether novelty can reverse reconsolidation impairments using a more widely used drug target and paradigm – beta-adrenergic antagonists and contextual fear conditioning. It has been shown that propranolol blocks reconsolidation of auditory fear memory when given after memory reactivation (Debiec and Ledoux, 2004) and reconsolidation of contextual fear memory of two different conditioning strengths and two reactivation delays (Taherian et al., 2014). It also blocks memory reconsolidation of passive avoidance and conditioned taste aversion (Villain et al., 2016). It can be administered to humans with good tolerance and has been shown to block fear memory reconsolidation in healthy adults (Kindt and van Emmerik, 2016; Thomas et al., 2017) and in patients with post-traumatic stress disorders (Brunet et al., 2008).

A widely used and well-characterized behavioral paradigm for reconsolidation studies, contextual fear conditioning, was used here to ensure the occurrence of memory destabilization and reconsolidation. It has been shown that a brief reactivation session can effectively lead to memory destabilization and reconsolidation (Nader et al., 2000). However, strong conditioning (Wang et al., 2009) or very short memory reactivation (Suzuki et al., 2004) can prevent memory reconsolidation from occurring. Thus, parameters in contextual fear conditioning for detecting memory reconsolidation were carefully chosen and described in methods.

On day 1, all rats received contextual fear conditioning (Fig. 6A). Four groups of rats (randomly assigned) showed similar levels of baseline freezing and post-footshock freezing (Fig. 6B, no significant difference between groups, all $F_{1,28}$ values were between 0.03 and 3.41, p values between 0.08 and 0.87). These 4 groups also showed similar levels of freezing during reactivation (Fig. 6C, all $F_{1,28} < 2.22$, p > 0.15) and the post-reactivation short-term memory test (Fig. 6D, all $F_{1,28} < 1.22$, p > 0.28).

At the post-reactivation long-term memory test (Fig. 6E), there was a significant drug effect ($F_{1,28} = 4.66$, p = 0.04). This was illustrated by significantly less freezing in the propranolol group, compared to the saline group when novelty was omitted after reactivation ($t_{14} = 3.34$, p = 0.005). Importantly, when novelty was introduced after reactivation, the propranolol group showed a similar level of freezing compared to the corresponding saline group ($t_{14} < 0.3$, p > 0.8). This significantly higher freezing in the propranolol group with novelty than the propranolol group without novelty ($t_{14} = 3.45$, p = 0.004) suggests that novelty can reverse the reconsolidation impairments caused by propranolol.

Similar drug and novelty effects were observed at 7 days later in the conditioning chamber (Fig. 6F, $F_{1,28} = 8.77$, p = 0.006). Without novelty after reactivation, there was less freezing in the propranolol group, compared to the saline group $t_{14} = 3.07$, p = 0.008). However, the propranolol group and saline group with novelty after reactivation froze at a similar level ($t_{14} < 1.3$, p > 0.22). The propranolol group with novelty froze significantly more than the propranolol group without novelty ($t_{14} = 2.96$, p = 0.01). This suggests that the reversal effect of novelty persisted for 1 week.

When rats were tested in a second context, they all showed significantly less freezing in the context in which footshocks had not been presented (Fig. 6G, $F_{1,31} = 90.15$, p < 0.001). There was no difference between groups (all $F_{1,28} < 3.73$, p > 0.05). This result suggests that the fear memory was specific to the conditioned context and the animals did not show generalized fear.

4. Discussion

4.1. Behavior tagging and capture

Current findings first demonstrate that introducing novelty after memory reactivation facilitated persistence of appetitive spatial memory. Second, protein-synthesis inhibitors, but not zif268 antisense, impaired the reconsolidation of the appetitive spatial memory. Third, beta-adrenergic antagonists impaired reconsolidation of contextual fear memory and introducing novelty after memory reactivation reversed this impairment.

Synaptic tagging and capture studies show that strong stimulation can facilitate the persistence of potentiation of a weakly stimulated pathway whether the strong stimulation is delivered before or after the weak one (Frey and Morris, 1997). Similarly, novelty can facilitate the persistence of spatial memory whether it is introduced before or after the memory encoding (Wang et al., 2010; Moncada and Viola, 2007) and whether it is before or after the memory reactivation (current study). This symmetric feature in timing is different from the standard memory modulation theory that focuses on interventions (e.g. epinephrine or glucocorticoids) after memory encoding (Roozendaal and McGaugh, 2011). There are also different time windows relating to the regulation of memory persistence through behavioral tagging and capture or through standard memory modulation. A time window of 30–60 min before or after memory encoding works well for behavioral tagging and capture to occur (Wang et al., 2010; Moncada and Viola, 2007). However, novelty does not improve memory persistence when given immediately after memory encoding (Moncada and Viola, 2007). In standard memory modulation, epinephrine given immediately after encoding works effectively in improving spatial memory in the water-maze (Hatfield and McGaugh, 1999) or object-location memory (Jurado-Berbel et al., 2010). The difference in temporal symmetry and time window may suggest multiple routes to modulate memory.

4.2. Memory of interest (MOI) and memory-modulating events (MMEs)

To understand the framework in behavioral tagging and capture, it is important to clearly identify MOI and MMEs. In the current study, exploration in a novel box was consistently used as a MME, which is effective for promoting an array of contextual or spatial memories. For example, it has been shown to promote the persistence of spatial appetitive memory (Wang et al., 2010) and the memory of weakly trained inhibitory avoidance (Moncada and Viola, 2007), object recognition, and contextual fear memory (Ballarini et al., 2009). Other types of novelty, such as novel tastes, can be used as an MME to promote the memory persistence of conditioned taste aversion (Ballarini et al., 2009) or latent inhibition (Merhav and Rosenblum, 2008).

Memory of object recognition can be used as an MOI instead of an MME. For example, Cassini et al. (2013) used spontaneous object recognition as an MOI and reconsolidation of contextual fear memory as a MME. They asked whether reactivation and reconsolidation of an MME during encoding and consolidation of an MOI can facilitate the persistence of the MOI. Notably, this is different from our approach of facilitating the persistence of the MOI through introducing an MME at reactivation and reconsolidation of MOI (Figs. 2 and 3). They found that reconsolidation of contextual fear memory around weak training of object recognition could facilitate the persistence of the object place memory. This study potentially implies that reconsolidation provides plasticity-related proteins or engages plasticity-related processes (Redondo and Morris, 2011) that can be captured by an MOI and lead to longer memory persistence. In contrast, the current study suggests that reactivation of an MOI engages a second round of the tagging process that can capture plasticity-related products or interact with the plasticity-related processes originated from the MME.

4.3. Novelty as a memory-modulating event

Brief exploration in a novel open field has been commonly shown to improve persistence of the MOI in various types of memory as described above. A common feature of these MOIs is that they all involve encoding spatial or environmental information, a process that likely
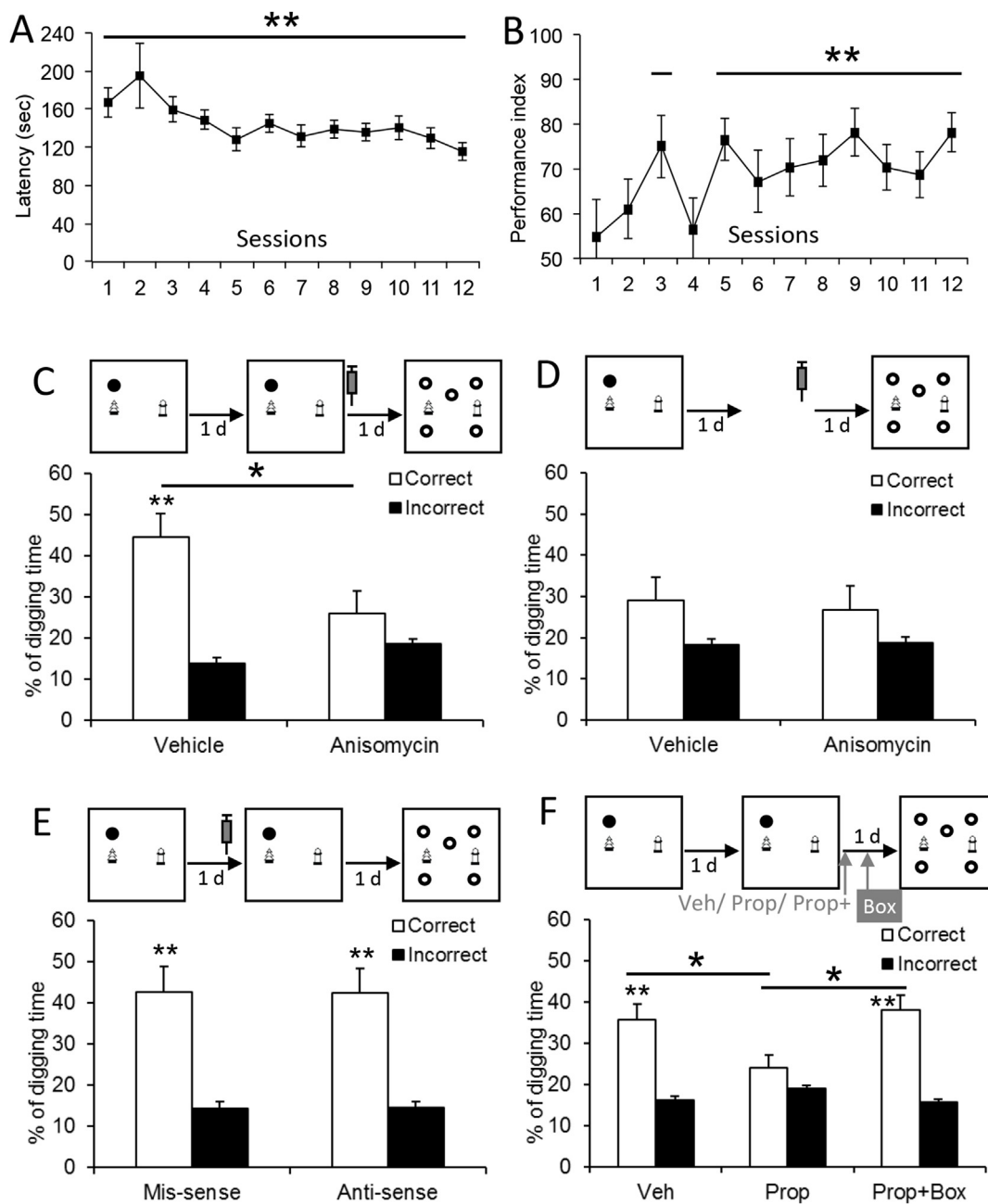
Fig. 5. Protein synthesis inhibitor, but not zif268 antisense, in the dorsal hippocampi impaired the reconsolidation of spatial appetitive memory. (A) The latency for rats to retrieve food rewards was significantly reduced over the 12 training sessions (**p < 0.005, indicating a significant linear trend). (B) The performance index was significantly above chance (which was 50) during training session no. 3, 5–12 (**p < 0.05). (C) Behavioral procedures on top show rewarded encoding (a filled circle) and reactivation in the event arena followed by a probe test with 5 non-rewarded sandwells (open circles). To ensure good memory on the day after encoding for examining drug-induced impairment on reconsolidation, a rewarded trial was used for memory reactivation followed immediately by protein synthesis inhibitor, anisomycin, or vehicle infusions. Animals showed significantly less percentage of time digging at the correct location after anisomycin treatment (*p < 0.05, compared to the vehicle condition). The correct digging percentage was significantly above chance in the vehicle condition (**p < 0.005, compared to 20%). (D) Behavioral procedures on top show rewarded encoding (a filled circle) and no reactivation before a probe test with 5 non-rewarded sandwells (open circles). The correct digging percentage was not significantly above chance in either vehicle or anisomycin condition and there was no significant drug effect. (E) Behavioral procedures similar to Fig. 5C were used except that zif268 anti-sense of or mis-sense was infused 90 min before memory reactivation. There was no significant difference between conditions and the correct digging percentage was significantly above chance in both treatments (**p < 0.005, compared to 20%). (F) Behavioral procedures similar to Fig. 5C were used except that propranolol (prop) or vehicle (veh) was systemically injected immediately after reactivation. A third condition involved exploration in a novel box at 30 min after prop injection. Correct digging was significantly above chance in veh and prop with box conditions (**p < 0.005, compared to 20%). Correct digging in these 2 conditions was significantly higher than in the prop only condition (*p < 0.05). Data = mean ± s.e.m.; n = 16.

engages the hippocampus. Another important process in memory is extinction, which also involves the hippocampus (Farinelli et al., 2006; Maren et al., 2013). When extinction of a learned memory is used as an MOI, novel open field exposure can also facilitate consolidation of

extinction (Furini et al., 2014).

Moreover, spatial novelty is effective at reversing memory reconsolidation impairment due to adrenergic blocker (Fig. 6), memory encoding impairment due to dopamine receptor antagonist (Wang
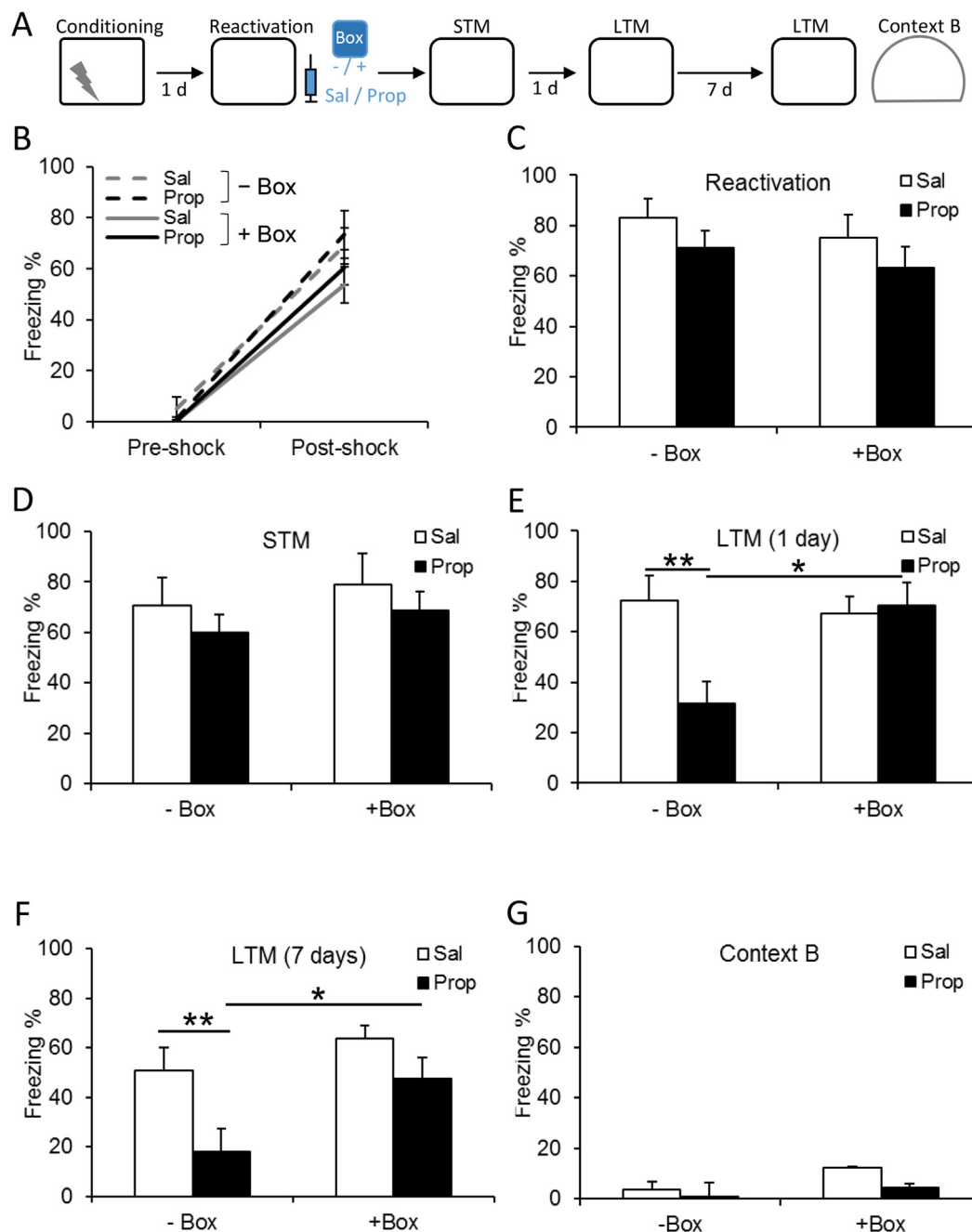
Fig. 6. Exploration in a novel box, introduced after memory reactivation, reversed propranolol-induced reconsolidation impairment of contextual fear memory. (A) Behavioral procedures showed contextual fear conditioning on day 1, re-exposure to the same context for memory reactivation followed by drug injection (saline, sal, or propranolol, prop) with or without exploration in a novel box on day 2, a short-term memory (STM) test at 4 h later, a long-term memory (LTM) test on day 3, and another LTM test and a test in a non-conditioned context B on day 10. (B) Percentage of time rats showed freezing during training before and after the delivery of footshock. All 4 groups showed comparable pre-shock and post-shock freezing during conditioning. (C) There was no significant difference of freezing among 4 groups during memory reactivation before drug injection and novel box exposure. (D) There was no significant difference of freezing among 4 groups on the STM test. (E, F) Propranolol, given after memory reactivation in the no box group, significantly impaired freezing at 1-day (E) and 7-day (F) post-reactivation LTM tests. Exploration in a novel box reversed the impairment by propranolol. (G) Freezing level in the non-conditioned context B was very low and there was no significant difference among 4 groups. Data = mean ± s.e.m.; n = 8 per group.

et al., 2010), memory consolidation impairment due to protein synthesis inhibitors (partial effect in Moncada and Viola, 2007), or memory consolidation impairment following aversive footshocks (Almaguer-Melian et al., 2012). However, not all kinds of novelty promote contextual or spatial memory. For example, novel object exploration that sufficiently produces long-term memory of object recognition does not facilitate persistence of spatial appetitive memory (Salvetti et al., 2014).

Novelty may sometimes result in an improvement of non-rewarded encoding but this effect is borderline. It is likely that non-rewarded encoding may sometimes trigger sufficient synapses and the hypothetical synaptic tagging mechanism, but sometime does not. One potential behavioral factor contributing to this difference is the duration that animals spend spontaneously exploring the arena. In both previous (Salvetti et al., 2014) and current studies, a non-rewarded trial stops when an animal voluntarily returns to the start box after free

exploration of the arena and sandwell (without reward), which does not involve the experimenter removing the animal after a set time limit and placing back in the start box, thus avoiding unexpected stress. As mentioned in the results, the rats in the current study spent less time in the arena in a non-rewarded trial than in the previous study. It is possible that the longer the animals spend spontaneously exploring the arena with the sandwell, the more synapses and cells are activated and thus, the probability that novelty enhances memory increases. In support of this concept, findings show that the longer exploration in a context, the more activity-related cytoskeleton-associated protein (Arc)-positive cells in CA1 of hippocampus are observed (Pevzner et al., 2012). The number of Arc-positive cells in CA1 triggered by exploration is also positively correlated to the number of laps the animals run in a rectangular track (Miyashita et al., 2009).

When the location encoded on the previous day and the new location just visited prior to novelty (i.e. the different condition in Fig. 4C) do not match, a conflicting situation arises. In these cases, if only the new location is processed before novelty exposure, without reactivating the location from the previous day, we would expect the memory of this location to be significantly better than chance, which is not the case. This implies that the information from the previous day is reactivated. However, it is conceivable that only partial sets of synapses or cells representing the previous location are active and hence novelty is insufficient to facilitate the original memory either. Future research is needed for understanding how conflicting information is processed during memory reactivation and how it affects behavioral decision during long-term memory recall.

Neural transmission involved in novelty for facilitating memory persistence has been investigated previously. Using pharmacological approaches, dopamine transmission through D1/D5 receptors and protein synthesis were shown to be required for a novel box to facilitate the persistence of an MOI (Moncada and Viola, 2007; Wang et al., 2010). The role of noradrenergic receptors in novelty is, however, less definite. While a beta-adrenergic receptor antagonist was shown to impair the novelty effect in facilitating the memory persistence of weak inhibitory avoidance (Moncada et al., 2011), another study that used a spatial appetitive memory similar to the current study did not support this (Takeuchi et al., 2016). Notably, propranolol in experiment 3 was given after memory reactivation and before novelty. The drug was effective in impairing memory reconsolidation but not in affecting the novelty effect (Fig. 6). If beta-adrenergic receptors were required for novelty, the reverse of memory reconsolidation impairment would not be seen. One difference between these findings is the target of drug infusion. It is possible that beta-adrenergic receptors in the dorsal dentate gyrus (Moncada et al., 2011) are selectively involved in the novelty effect on memory modulation. Targeting the wider dorsal hippocampi that also includes dentate gyrus or applying drug systemically perhaps does not inhibit dentate gyrus substantially enough to affect novelty. This will require future studies to disambiguate.

4.4. Factors regulating memory reactivation and reconsolidation

Reconsolidation has been widely studied (Tronson and Taylor, 2007) and there are constraints on whether reconsolidation occurs after memory reactivation. For example, the length of memory reactivation is critical. Short memory reactivation can engage reconsolidation while long reactivation can engage extinction (Lee et al., 2004; Mamiya et al., 2009). In the current study, reactivation was either rewarded (Figs. 2C and 3) or very brief (Fig. 2A and C, Fig. 6); hence, extinction was unlikely to occur.

In Experiment 3, a brief, 90 s re-exposure to the conditioned context was sufficient to reactivate the fear memory and lead to reconsolidation impairment due to propranolol (Fig. 6C). This is largely consistent with reconsolidation studies using brief exposure to the conditioned stimuli. For example, a brief replay of the conditioned tone (around 30 s) is commonly used to reactivate fear memory and render the memory

sensitive to application of amnesic agents (e.g. Wang et al., 2009; Huynh et al., 2014; Lopez et al., 2015). This is different from a previous study showing that 90 s reactivation exposure was insufficient to improve memory persistence of object recognition memory (Cassini et al., 2013). This difference echoes the importance of differentiating MOI and MME. As shown in this study, when contextual fear memory is the MOI, a brief reactivation does engage the process for reconsolidation that is sensitive to propranolol. When contextual fear memory is used as MME, a brief reactivation (90 s) may not trigger sufficient plasticity-related products or processing for capturing, and as such a longer reactivation (3 min) is required (Cassini et al., 2013). Indeed, a sufficiently novel or longer event (5 min) as a MME is typically required for behavior tagging and capturing to occur (Moncada et al., 2015).

Another constraint on reconsolidation is the strength of training. In auditory fear conditioning, when the number of cue-footshock pairings are increased to ten, protein synthesis inhibition in the amygdala after memory reactivation does not lead to impairment of long-term memory (Wang et al., 2009; Holehonnur et al., 2016). This suggests that very strong fear memory does not destabilize easily after memory reactivation and hence does not engage protein synthesis-dependent reconsolidation. The receptor candidates, such as GluN2B or GluN2A/GluN2B ratio, for determining memory destabilization has been proposed (Wang et al., 2009; Holehonnur et al., 2016). Pharmacologically regulating receptors during memory reactivation also elucidates mechanisms underlying memory destabilization (Hong et al., 2013). In the current study, a standard, not very strong, conditioning protocol was used and no constraint on reconsolidation was observed (Fig. 6B).

For reconsolidation of spatial memory, it has been shown in the watermaze that protein synthesis inhibition in the hippocampus impairs reconsolidation of delayed-matching-to-place memory, but not reference memory (Morris et al., 2006). Other studies show that reconsolidation of reference memory can be impaired by mRNA synthesis inhibition (Da Silva et al., 2008). Moreover, reference memory formed after less training trials can be impaired by protein synthesis inhibition in the hippocampus (Kim et al., 2011, review see Wang and Morris, 2010). The spatial appetitive paradigm here shares a comparable learning principle of spatial updating across sessions with the delay-matching-to-place task in the watermaze. Similar to the finding in watermaze (Morris et al., 2006), protein synthesis inhibitors, given in the hippocampus after memory reactivation, impair the persistence of matching-to-place memory. This finding also rules out any concern of memory strength in constraining reconsolidation in this paradigm despite prolonged training. Zif268 anti-sense in the hippocampus did not lead to memory impairment in Fig. 2C. This lack of effect is inconsistent with previous findings showing zif268 that antisense in the hippocampus impaired reconsolidation of conditioned contextual fear (Lee et al., 2004; Trent et al., 2015) or in amygdala in impairing conditioned drug-seeking behavior (Lee et al., 2005) or conditioned place preference (Théberge et al., 2010). The concentration, volume of injection, and the production of oligodeoxynucleotides were carefully chosen to be consistent with previous studies. It remains possible that more substantial knockdown of Zif268 protein expression (e.g. more than 60%, Lee et al., 2004, 2005) or multiple injection sites to also cover the intermediate (Kenney and Manahan-Vaughan, 2013) and/or ventral hippocampus (Wang et al., 2012b) is required to block memory reconsolidation in this paradigm. Alternatively, whether zif268 is required for reconsolidation of memories that are not based on classical conditioning requires future investigation.

Targeting memory reactivation has been shown to provide a promising pathway for treating post-traumatic stress disorders by interfering or blocking the reconsolidation process (Dunbar and Taylor., 2017; Brunet et al., 2008). Contrary to weakening aversive memory, the time window of reactivation and reconsolidation can be used for improving desired memory. Using pharmacological and behavioral approaches, current findings establish the method of facilitating memory persistence through behavioral tagging and capture during memory

reconsolidation. This model also provides new avenues for memory improvement and for understanding its underlying neurobiological mechanism.

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