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Effects of the Protein Synthesis Inhibitor Cycloheximide or the β-Adrenergic Antagonist Propranolol on Reconsolidation of Spatial Memory or Auditory Fear Conditioning, Respectively, in Adolescent Rats

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Memory reconsolidation, or the concept that an old previously consolidated memory must undergo reconsolidation following retrieval from long-term memory, has received considerable attention over the last decade. Relatively little evidence, however, has examined this phenomenon in developmentally immature organisms, and even fewer studies have considered a state-dependent retrieval explanation for amnesias associated with disruption of reconsolidation processes. Here we report the results of two experiments using adolescent rats and a state-dependent design. In Experiment 1, animals acquired a spatial memory in the Morris water maze, but failed to show any sign of reconsolidation impairment or state-dependency using the protein synthesis inhibitor cycloheximide. In Experiment 2, animals were given auditory fear conditioning, but also did not show any evidence of a reconsolidation impairment or state-dependency using the β-adrenergic antagonist propranolol, although there was evidence of extinction. The results of these studies are discussed in terms of the strength of the memory trace and its subsequent susceptibility to amnestic treatments.

The existence of an immediate post-learning consolidation phase during which newly learned information undergoes a time limited period of processing has been well established (Dudai, 2004). During consolidation, exposure to certain drugs, head trauma, or other events may impair processing, thus reducing the subsequent strength of, accessibility to, or even the establishment of the long-term memory. Historically, the consolidation hypothesis posits that once consolidation of the new information has ended, the memory becomes immune to treatments or conditions that might have altered the strength of the memory during the consolidation phase. Over the last 10 years many studies have challenged this view, demonstrating that the retrieval of an old memory (previously consolidated) returns it to a malleable state where it must undergo reconsolidation (see Flint et al., 2010 for a review). The purpose of the experiments presented here was to examine this reconsolidation process in immature adolescent rats, a relatively understudied group, using tasks and treatments which have provided evidence of reconsolidation in adult animals.

As mentioned above, evidence of a retrieval-dependent reconsolidation process has been repeatedly exhibited over the last decade. Studies have demonstrated memory reconsolidation using a wide range of species including humans (Walker, Brakefield, Hobson, & Stickgold, 2003), rats (Flint & Marino, 2007; Flint, Valentine, & Papandrea, 2007), mice (Blundell, Kouser, & Powel, 2008), chicks (Litvin & Anokhin, 2000), ewe (Perrin et al., 2007), *drosophila* (Lagasse, Devaud, & Mery, 2009), snails (Gainutdinova et al., 2005), and others. Given this variety, reconsolidation appears to be a very generalizable phenomenon, and not surprisingly, has been demonstrated with a wide variety of learning/memory paradigms (see Flint et al., 2010 for review). For example, Flint, Valentine, and Papandrea (2007) used the Morris water maze to examine protein synthesis-dependent memory reconsolidation in male and female rats. They found that cycloheximide administered at the time of memory reactivation significantly disrupted memory reconsolidation and that this effect was more pronounced in female animals, which typically perform more poorly in the water maze than males (Jonasson, 2005). Using an auditory fear conditioning paradigm, Debiec and LeDoux (2002) disrupted reconsolidation of the long-term memory trace in rats by blocking noradrenergic receptors in the amygdala with propranolol. Many other such examples exist, but these two are particularly important to the experiments reported here, as they demonstrate that the protein synthesis inhibitor cycloheximide disrupts long-term spatial memory reconsolidation in the Morris water maze and that the β-adrenergic receptor antagonist propranolol disrupts memory reconsolidation of auditory fear conditioning in adult rats.

One caveat of many consolidation and reconsolidation studies which researchers often fail to consider is the possibility of a state-dependent retrieval failure explanation for the poor performance seen during the retention test (see Baumbauer, Anderson, & Riccio, 2002 for review). In state-dependency, animals learn, consolidate, or potentially reconsolidate information in a particular drug state. On a subsequent retention test, animals in a normal state (i.e., having now received a saline or placebo injection at testing) perform poorly, a result that might be interpreted as a disruption of learning or consolidation by the drug at training or disruption of reconsolidation at the time of memory reactivation. However, animals that are administered the drug at the time of the retention test may show evidence of good retention, indicating that the drug state was likely encoded with the information at training, and thus likely served as a cue which facilitated retention performance during

the test. Studies of memory reconsolidation rarely consider the possibility that the reconsolidation 'impairing' effects of a drug might actually represent a state-dependent effect, where the drug state is encoded during reconsolidation and poor performance during the subsequent retention test is actually a retrieval failure associated with a reconsolidation/testing mismatch in internal cues. Indeed, recent unpublished data from our lab indicates that the NMDA antagonist MK-801 may produce reconsolidation impairments for passive avoidance conditioning in adolescent rats, but that the reconsolidation impairment is actually state-dependent, such that the re-administration of MK-801 at testing results in good retention performance for those animals that had it during reconsolidation. This finding is consistent with state-dependent effects of MK-801 on consolidation of passive avoidance conditioning in adult rats (Ceretta, Camera, Mello, & Rubin, 2008; Harrod, Flint, & Riccio, 2001). A second purpose of the experiments reported here was to explore potential state-dependent reconsolidation effects for cycloheximide and propranolol in adolescent animals.

Of particular importance to the present studies are the recent results indicating that reconsolidation processes are intact in immature rats. Languille, Gruest, Richer and Hars (2008) examined taste/odor aversion memory in 3-, 10-, and 18-day-old rats. Their results indicated that the protein synthesis inhibitor anisomycin produced disruptions in both consolidation and reconsolidation processes, however, the results were not consistent across age groups. The pattern of results indicated that the amount of time necessary for protein synthesis-dependent memory decreases with age. In a similar study, Languille et al. (2009) reported disruption of consolidation and reconsolidation for a taste aversion memory in 3-day-old rats with a mitogen-activated protein kinase kinase inhibitor (SL327). Evidence of memory reconsolidation has also been reported in studies of human infants (Galluccio, 2005; Galluccio, & Rovee-Collier, 2005). The results of these studies suggest that the neurobiological mechanisms for at least some forms of long-term memory are intact very early in postnatal development.

Given that both 3-day-old and adult rats have exhibited evidence of memory reconsolidation, it may seem logical to generalize these findings to age groups in between such as adolescent animals. However, such generalizations should be made cautiously. Extensive research on adolescent animals by Norman and Linda Spear have indicated that adolescence represents a unique developmental period during which animals may be differentially affected by drugs and may behave differently from adults in some test of memory and cognition (Brasser & Spear, 2004; Land & Spear, 2004; Rajendran & Spear, 2004). Thus, the general purpose of these experiments was to examine memory reconsolidation processes in adolescent animals. Experiment 1 hypothesized that the protein synthesis inhibitor cycloheximide would disrupt reconsolidation of spatial memory in the Morris water maze in adolescent rats. In Experiment 2 it was similarly hypothesized that the β-adrenergic antagonist propranolol would disrupt memory reconsolidation of auditory fear conditioning in adolescent rats.

Experiment 1

Spatial learning and memory is commonly assessed in rats using the Morris water maze (Morris, 1981). Learning proceeds relatively quickly across days with small blocks of trials and has been shown in infant animals as well (Carman & Mactutus, 2001). A number

of studies have now demonstrated memory reconsolidation effects in adult animals using the water maze and protein synthesis inhibitors such as cycloheximide (Flint et al., 2007) and anisomycin (Artinian, De Jaeger, Fellini, de Saint Blanquat, & Roullet, 2007; Morris et al., 2006; Rossato, Bevilaqua, Medina, Izquierdo, & Cammarota, 2006). The purpose of this experiment was to examine the effects of cycloheximide on memory reconsolidation in adolescent rats using the Morris water maze and to explore the possibility of state-dependent retention.

Method

Subjects

Forty naïve male Sprague-Dawley rats (Hilltop Lab Animals, Inc.) served as subjects. All animals were allowed access to food and water *ad libitum* and were housed in standard opaque Plexiglas cages in groups on a 15:9 hour reversed light:dark cycle. All training took place during the animal's dark phase. Animals were weaned at 21 days old and were 29 days old at the onset of training. Animals were handled for approximately 2 minutes each day for 3 days before the beginning of the study.

Apparatus & Materials

The plastic tank used for the Morris water maze was obtained from Terracon Corporation (Holliston, MA). The tank was painted black and measured 124.5 cm in diameter and 75 cm deep. Water was added to the tank until the water surface was 17 cm from the top edge, providing an animal in the water with a good view of the various extra-maze cues. The tank was positioned approximately in the center of a small research room with a door at one end, a window on one wall, and unique decorations on the other walls. The room was illuminated with a 300 watt halogen lamp placed on the floor and directed toward the adjacent wall and ceiling. This light was sufficient to illuminate the entire room, but did not provide any glare on the water surface which might disrupt the digital tracking system. The maze was divided into four quadrants, NE, NW, SE, and SW. The researcher was always positioned at the S end of the apparatus and a round black platform measuring 11.4 cm in diameter was located in the center of the NE quadrant 1.5 cm below the surface of the water. A standard laboratory stopwatch was used to time each training trial. A computer with the animal tracking software AnyMaze (Stoelting, Inc., Wood Dale, IL) was connected to a digital camera for digitizing each animal's behavior during the probe tests. The computer was also used to generate 68 dB of white noise to mask potential background disturbances during training and testing. Saline (85% sterile saline, Sigma Chemical, St. Louis, MO) or 1 mg/kg of the protein synthesis inhibitor cycloheximide (Oxoid, Ltd., Cambridge, UK) was administered subcutaneously in the nape of the neck at a volume of 1 ml/kg bodyweight.

Procedure

Animals were trained in groups of four, such that each animal in the group completed the first training trial, followed by the second animal's first trial, and so on until all four animals completed their first trial before beginning trial two. Using this arrangement the intertrial interval was maintained at six to eight minutes for each animal. Animals received

four training trials each day for three consecutive days. A single training trial involved placing the animal into the water maze at the N, S, E, or W location of the water maze facing the side of the tank and allowing it to swim and explore the tank for up to 120 seconds. If the animal discovered the submerged platform prior to the end of the trial, the researcher stopped the watch, recorded the latency to the platform, and allowed the rat to observe its surroundings for 30 seconds before removing it, drying it off, and returning it to its home cage. If the animal failed to discover the submerged platform before the end of the trial the researcher physically guided the animal to the platform, recorded a training latency of 120 seconds, and gave the animal 30 seconds to observe its surroundings. The placement location of the rat into the water maze was the same for each animal, but varied from trial to trial in a pseudorandom order such that placement location was not repeated more than twice in a single training day and never repeated two trials in a row. This approach was adopted so that animals would be less likely to learn the location of the submerged platform using a motor strategy (i.e., turn left to find the platform), and more likely that they would learn the location of the platform based on extra-maze cues.

After completing the training trial, animals were given a 48 hour rest period. When the protocol resumed all animals were given a single reactivation treatment followed immediately by a subcutaneous injection. Half of the animals received an injection of saline and half received cycloheximide. Prior to the reactivation trial, the submerged platform was removed from the tank and the water height was adjusted so that it was the same as it was during training, affording animals the same view of the extra-maze cues. For the reactivation trial, animals were placed into the water maze at the S end and were given 120 seconds to explore the maze. A single non-reinforced trial was used for memory reactivation since prior work has indicated that a single trial reactivates the training memory and initiates reconsolidation, whereas more extensive non-reinforced trials activate the neurobiological mechanisms of extinction and lead to extinction of the acquired behavior (Berman & Dudai, 2001; Mamiya et al., 2009; Suzuki et al., 2004). Following the reactivation trial and injection, animals were given another 48 hour rest period before the retention test.

On the day of the retention tests, animals were administered a subcutaneous injection of saline or cycloheximide such that half of the saline group from the reactivation day now received saline again while the other half received cycloheximide, and half of the cycloheximide group from the reactivation day received cycloheximide again and the other half received saline. This standard state-dependent design produced four drug groups based on the drug received at reactivation and the drug received at testing (saline/saline, saline/cycloheximide, cycloheximide/cycloheximide, and cycloheximide/saline). Injections were administered 20-25 minutes prior to the first testing trial. All animals were given three retention probe tests in groups of four in the same manner as the reactivation trial. Researchers remained blind to all drug conditions until data collection was completed.

Results

The latency to the platform during training was analyzed using a day by trial (3x4) repeated measures analysis of variance (ANOVA). Results revealed significant main effects of day [$\eta_p^2 = .70$; Huynh-Feldt correction $F(1.74,62.77) = 85.16$, $p < .001$], and trial [$\eta_p^2 = .40$; Huynh-Feldt correction $F(2.76,99.46) = 23.63$, $p < .001$], and a significant day by trial interaction [$\eta_p^2 = .11$; Huynh-Feldt correction $F(4.47,160.94) = 4.50$, $p = .001$] (see

Figure 1: *Mean latencies to reach the stationary submerged platform during training in the Morris water maze across trials for each of the three days of training (groups have been collapsed).*

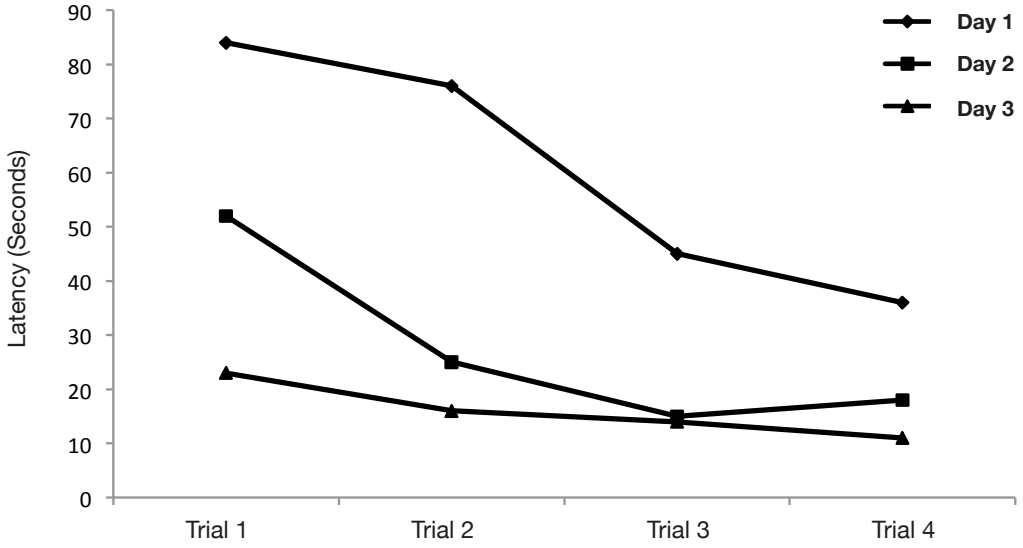
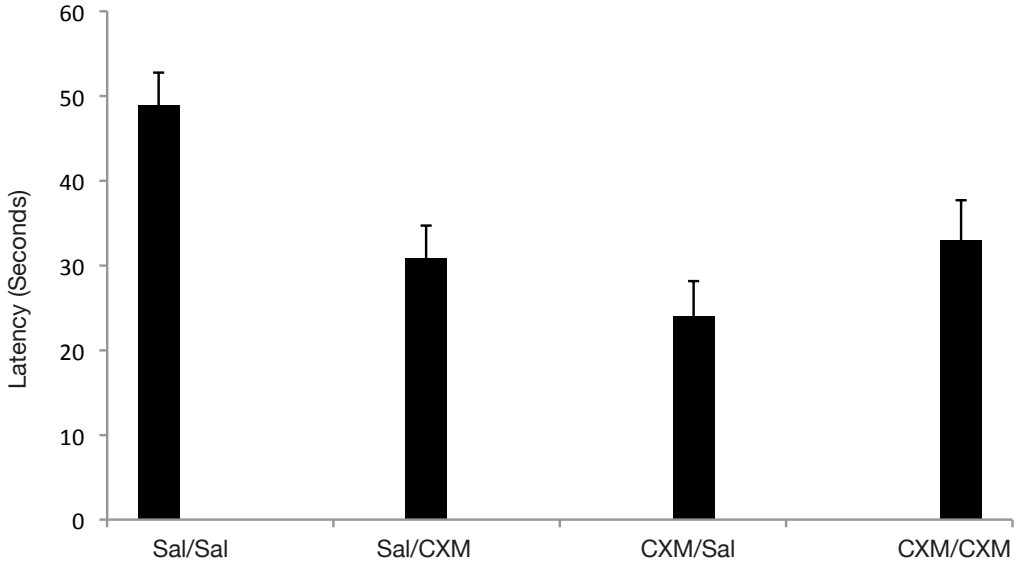


Figure 1).

Following the memory reactivation trial, three animals were eliminated from the study, one because of an injection error and two because they failed to cross over the platform zone at all during the reactivation trial. A one-way ANOVA for the total distance traveled during the first test trial did not reveal any significant differences among the groups [$\eta_p^2 = .10$; $F(3,37) = 1.24$, $p > .05$; power = .30]. The same result was found for the overall average speed during the first test trial [$\eta_p^2 = .16$; $F(3,37) = 2.12$, $p > .05$; power = .49] suggesting that there were no adverse effects of cycloheximide on the animals sensorimotor ability in the water maze.

Group by test trial (4x3) mixed ANOVAs for the dependent measures during the three probe tests did not reveal any significant effects for the number of entries into the platform zone {group [$\eta_p^2 = .004$; $F(3,33) = .05$, $p > .05$; power = .06]; test trial [$\eta_p^2 = .01$; Huynh-Feldt correction $F(1.88,62.18) = .43$, $p > .05$; power = .12]; group by test trial interaction [$\eta_p^2 = .06$; Huynh-Feldt correction $F(5.65,62.18) = .69$, $p > .05$; power = .25]}, time spent in the platform zone {group [$\eta_p^2 = .05$; $F(3,33) = .54$, $p > .05$; power = .15], test trial [$\eta_p^2 = .002$; Huynh-Feldt correction $F(1.80,59.47) = .05$, $p > .05$; power = .06], group by test trial interaction [$\eta_p^2 = .09$, Huynh-Feldt correction $F(5.41,59.47) = 1.03$, $p > .05$; power = .35]}, the time spent in the NE quadrant where the platform was located {group [$\eta_p^2 = .04$; $F(3,33) = .74$, $p > .05$; power = .13], test trial [$\eta_p^2 = .03$; Huynh-Feldt correction $F(2,66) = .37$, $p > .05$, power = .22], group by test trial interaction [$\eta_p^2 = .03$; Huynh-Feldt correction $F(6,66) = .33$, $p > .05$; power = .14]}, and the distance traveled in the NE zone where the platform was located {group [$\eta_p^2 = .11$; $F(3,33) = 1.30$, $p > .05$; power = .31], test trial [$\eta_p^2 = .05$; Huynh-Feldt correction $F(2,66) = 1.60$, $p > .05$; power = .33], group by test trial interaction [$\eta_p^2 = .03$; Huynh-Feldt correction $F(6,66) = .31$, $p > .05$; power = .13]}. However, the latency to the platform zone measure during the probe tests did reveal a significant main effect of group [$\eta_p^2 = .43$; $F(3,28) = 7.14$, $p = .001$]. Post-hoc Fisher's LSD tests for pairwise comparisons revealed that the saline/saline group had significantly longer

Figure 2: *Mean latency to the platform zone for each group collapsed across the three probe tests. Error bars represent the standard error of the mean.*



latencies to the platform zone than the cycloheximide/saline [MD = 24.83, p < .001], saline/cycloheximde [MD = 18.05, p = .003], and cycloheximide/cycloheximide groups [MD = 15.94, p = .01] (see Figure 2). There was no effect of test trial [η_p^2 = .01; Huynh-Feldt correction F(2,56) = .14, p > .05; power = .07] or interaction [η_p^2 = .30; Huynh-Feldt correction F(6,56) = 1.24, p > .05; power = .45].

Experiment 2

Prior research using the central acting β-adrenergic receptor antagonist propranolol has shown that this receptor plays an important role in reconsolidation of auditory fear conditioning (Debiec & LeDoux, 2004) as well as in decreasing anxiety in the open field (Angrini, Leslie, & Shephard, 1998) and light-enhanced startle (Walker & Davis, 2002). Propranolol is also available for use in humans and has been shown to reduce stage fright (Brantigan, Brantigan, & Joseph, 1982), test anxiety (Faigel, 1991), and contextual fear conditioning (Grillon, Cordova, Morgan, Charney, & Davis, 2004). The purpose of this experiment was to examine the effects of propranolol on memory reconsolidation and state-dependency for auditory fear conditioning in adolescent rats.

Methods

Subjects

Forty naïve male Sprague-Dawley rats (Hilltop Lab Animals, Inc.) served as subjects and were maintained under the same conditions described in Experiment 1. All training took place during the animal's dark phase. Animals were weaned at 21 days old and were 29 days old at the onset of training. Animals were handled for approximately 2 minutes each day for 3 days before the beginning of the study.

Apparatus & Materials

Training was carried out on the black (dark) side of a standard passive-avoidance chamber (Apparatus A, Ugo Basile, Italy). The sides of the chamber were constructed of black Plexiglas measuring 22.2 by 22.2 cm wide and 22.2 cm high. The floor was constructed of metal bars spaced 1 cm apart through which a 1 second 1.0 mA footshock could be delivered. During training the lid was left open and a 10 second tone could be delivered from a speaker positioned just above the chamber. This apparatus was maintained in a small testing room illuminated by a single 60 watt incandescent bulb.

Memory reactivation and testing took place in a refurbished operant conditioning chamber (Apparatus B). Two of the opposing walls of the apparatus were constructed of clear Plexiglas while the remaining two walls were made of aluminum. Walls measured 22.9 by 20.3 cm wide and 19.1 cm high. The floor was constructed of metal bars positioned 1.1 cm apart. The lid of the apparatus was left open and the same sound generator was used to provide the tone CS. Apparatus B was maintained in a separate room illuminated with overhead fluorescent lighting. In order to enhance the differences between the contexts, three drops of peppermint extract were placed onto the bedding beneath the floor of the apparatus at the beginning of each day and 68 dB of white noise was used to mask background disturbances.

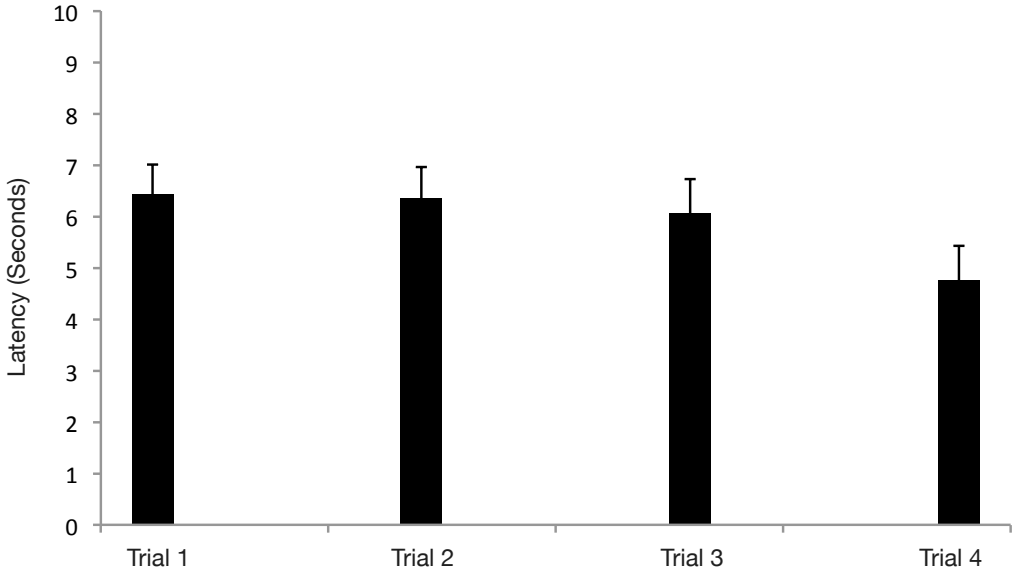
All injections were administered intraperitoneally at a volume of 1 ml/kg body weight. Propranolol (Sigma) was delivered at 10 mg/kg and 85% sterile saline (Sigma) was administered as a control.

Procedure. Animals were randomly assigned to one of four groups (n=10) using a state-dependent design. All animals were given two injections, one immediately following memory reactivation and a second shortly prior to the retention test. Researchers remained blind to the drug condition until all data were collected.

For training all animals were placed individually into Apparatus A for approximately two minutes. At the end of the two minutes a 10 second tone was presented which co-terminated with a 1 second footshock. This procedure was repeated every two minutes for a total of five trials. Following the last trial, the animal was removed from the apparatus, returned to its home cage, and the apparatus was thoroughly cleaned with a 10% ethanol solution.

On day 2, animals were placed individually into Apparatus B for 10 minutes after which they were returned to their home cage and the apparatus was thoroughly cleaned before the next animal. On day 3, animals were placed into apparatus B for two minutes at the end of which the tone was turned on for 10 seconds. By presenting the tone from training, this trial served to reactivate the training memory. No shock was administered. Immediately after the cue presentation, animals were removed and administered either saline or propranolol. Animals were given 48 hours rest before the retention test. Approximately twenty minutes prior to the retention test, each animal was administered either saline or propranolol in the traditional state-dependent manner such that four groups were created based on the treatment at reactivation and at testing (saline/saline, saline/propranolol, propranolol/propranolol, and propranolol/saline). For the retention test, animals were placed into Apparatus B for two minutes followed by a 10 second tone. The tone was then presented for 10 seconds every minute until it had been presented 4 times. The animal's behavior was recorded on video (Radio Shack) for subsequent analysis.

Figure 3: *Mean time spent freezing across the four test trials during which the CS (tone) was presented for 10 seconds each trial (groups have been collapsed). Error bars represent the standard error of the mean.*



Results

The time spent freezing during the reactivation cue and the four 10-second cue test trials was determined independently by two blind coders. Correlation coefficients between the coder's scores ranged from .81 for the first cue test to .99 for the forth cue test, and all correlations were highly significant (p's < .001). Given this high degree of interrater reliability, the rater's scores were averaged for subsequent statistical analyses.

A one-way ANOVA for the reactivation treatment did not reveal any differences among the groups [η_p^2 = .13; $F(3,33) = 1.47$, $p > .05$; power = .35], indicating that each group demonstrated the same level of conditioned fear. A mixed group by test trial ANOVA revealed a significant main effect of test trial [η_p^2 = .14; Huynh-Feldt correction $F(2.63,78.96) = 5.02$, $p < .005$] indicating that animals gradually extinguished some of their conditioned fear across the trials (see Figure 3). There was no main effect of group [η_p^2 = .04; $F(3,30) = .42$, $p > .05$; power = .12] or group by test trial interaction [η_p^2 = .12; Huynh-Feldt correction $F(7.90,78.96) = 1.41$, $p > .05$; power = .60].

Discussion

The general purpose of these experiments was to examine memory reconsolidation processes in adolescent rats using established protocols from studies of adult animals. In addition to this primary objective, the experiments were designed to test for a state-dependent explanation of memory impairments associated with treatments administered at the time of reconsolidation. The results failed to find any evidence of a cycloheximide-induced reconsolidation impairment in the water maze (Experiment 1) or a propranolol-induced reconsolidation impairment for auditory fear conditioning (Experiment 2). In both experiments, animals appeared to acquire the task well and maintain good retention.

In Experiment 1, animals showed clear evidence that they learned the location of the submerged platform across the training trials and days. The total distance traveled and overall average speed measures did not reveal any group differences on the first probe trial, suggesting that cycloheximide did not differentially affect behavior in any way that might be construed as amnesia. All of the dependent measures, with the exception of the latency to the platform zone measure, failed to reveal any main effects or interactions. Interestingly, the latency to the platform zone measure indicated that the saline/saline group performed significantly worse (i.e., longer latencies to the platform zone) than all of the other groups. It is not at all clear why such a result was found, especially since other well-accepted dependent measures of spatial memory for the water maze (e.g., number of crosses over platform zone and time spent in platform zone) did not reveal the same pattern of results. In comparison to the saline/saline group, one of the remaining groups also received saline at test, and another received cycloheximide at reactivation and testing, thus this significant group difference cannot be easily explained as a 'cycloheximide' effect or as a match or mismatch in internal drug state. Additional research replicating this finding will be necessary to determine whether or not this is a reliable effect or simply a false positive.

Given the strong evidence of spatial learning in Experiment 1, it is interesting that none of the dependent measures revealed any evidence of extinction across the three probe trials. The fact that there is no evident extinction suggests that initial learning and the strength of the memory trace was sufficiently strong so as to require substantially more probe tests before evidence of extinction would be revealed. The strength of the memory trace may be related to our failure to find any effects of cycloheximide, as we hypothesize in more detail below.

The time spent freezing during acquisition in Experiment 2 was not recorded and as a result, we do not have data regarding the strength of learning as we did with Experiment 1. However, animals clearly learned to associate the CS with the US and displayed significant evidence of extinction during testing, as evidenced by a decrease in the time spent freezing across the test trials. Even though extinction began to develop, it was far from complete, as animals spent an average of approximately five seconds in an immobile state during the presentation of the CS, even after three previously non-reinforced CS presentations. The continuation of a high level of conditioned freezing suggests that the CS-US association was very well learned and maintained, which may have made the memory resistant to disruption with propranolol.

As implied above, the failure to impair reconsolidation in these experiments may be related to the strength of original learning and of the subsequent memory trace. In other words, the level of initial learning may have been strong enough to render the memory immune to the impairing effects of protein synthesis inhibition in Experiment 1 and to β-adrenergic blockade in Experiment 2. Such a conclusion is supported by a number of studies demonstrating that overtraining may offer protection from amnesia-inducing treatments (Flood, Bennet, Rosenzweig, & Orme, 1972; Flood, Rosenzweig, Bennet, & Orme, 1973; Flood, Bennet, Orme, & Rosenzweig, 1975ab; Gray & Meyer, 1981). In the present experiments, protocols were developed based on studies of reconsolidation from our lab and other's using the water maze and auditory fear conditioning. Since no studies have examined reconsolidation in adolescent animals, it is difficult to conclude with any certainty whether or not these protocols produced overtraining. However, the strong learning and memory evidenced by our animals suggests that overtraining may have been a factor in their resistance to the amnestic treatments.

In conclusion, the results of these two experiments suggest that well-established memories for spatial location in the water maze and for auditory fear conditioning in adolescent rats lead to their likely immunity to cycloheximide- and propranolol-induced reconsolidation impairment, respectively. These studies do not, however, rule out the possibility that such memories may sometimes be susceptible to such treatments, especially given that evidence of this has been documented in adult animals. It is more likely that the training parameters were such that the strength of the memory trace rendered it immune to disruption at reconsolidation. Additional studies in which the strength of conditioning or initial learning is reduced will help to reveal whether this is a procedural issue or an ontogentic one.

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