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POST-RETRIEVAL PROPRANOLOL TREATMENT DOES NOT MODULATE RECONSOLIDATION OR EXTINCTION OF ETHANOL-INDUCED CONDITIONED PLACE PREFERENCE

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Abstract

The reconsolidation hypothesis posits that established emotional memories, when reactivated, become labile and susceptible to disruption. Post-retrieval injection of propranolol (PRO), a nonspecific ß-adrenergic receptor antagonist, impairs subsequent retention performance of a cocaine- and a morphine-induced conditioned place preference (CPP), implicating the noradrenergic system in the reconsolidation processes of drug-seeking behavior. An important question is whether post-retrieval PRO disrupts memory for the drug-cue associations, or instead interferes with extinction. In the present study, we evaluated the role of the ß-adrenergic system on the reconsolidation and extinction of ethanol-induced CPP. Male DBA/2J mice were trained using a weak or a strong conditioning procedure, achieved by varying the ethanol conditioning dose (1 or 2 g/kg) and the number of ethanol trials (2 or 4). After acquisition of ethanol CPP, animals were given a single post-retrieval injection of PRO (0, 10 or 30 mg/kg) and tested for memory reconsolidation 24 h later. Also, after the first reconsolidation test, mice received 18 additional 15-min choice extinction tests in which PRO was injected immediately after every test. Contrary to the prediction of the reconsolidation hypothesis, a single PRO injection after the retrieval test did not modify subsequent memory retention. In addition, repeated post-retrieval administration of PRO did not interfere with extinction of CPP in mice. Overall, our data suggest that the ß-adrenergic receptor does not modulate the associative processes underlying ethanol CPP.

Keywords: Extinction; Reconsolidation; Conditioned Place Preference; Propranolol; Alcohol; Mice

1. Introduction

The memory consolidation hypothesis posits that memories, while initially fragile, stabilize over time becoming permanent through a process that requires new gene expression (Davis and Squire, 1984; McGaugh, 2000). During the initial phase of consolidation, the memory trace is labile and can be disrupted by either pharmacological or physical manipulations such as amnesic agents, seizure, brain injury or trauma (Cahill et al., 2000; Dudai, 2004; McGaugh, 2000)[·]. However, once the consolidation process is complete, stable memories are permanently stored and insensitive to interference (McGaugh, 2000). During the last few decades, many investigators have challenged the idea of a permanent memory store (Pedreira et al., 2002; Przybyslawski, et al., 1999; Riccio et al., 2006). Recent findings have revitalized the debate that memory retrieval involves a new phase of memory lability in which the memory trace is returned to an active state vulnerable to disruption (Nader et al., 2000; Sara, 2000). Accordingly, the reconsolidation hypothesis states that old, well-consolidated memories return to a labile stage when reactivated and subsequently require de novo protein synthesis for their reconsolidation (Alberini et al., 2006; Dudai, 2000; Nader et al., 2000; Sara, 2000).

Retrieval of the original memory is the key event that initiates reconsolidation. For example, in a conditioned place preference (CPP) procedure, a conditioned stimulus (CS, such as a tactile cue) is repeatedly paired with an unconditioned stimulus (US, such as cocaine). After several pairings between the CS and the US, preference for the CS is typically assessed during a retrieval test in the absence of the US. This retrieval test would presumably engage the memory

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· Abbreviations: Propranolol (PRO); Conditioned Place Preference (CPP); ß-adrenergic receptor (ß-AR); Conditioned Stimulus (CS); Unconditioned Stimulus (US); Intraperitoneally (IP); Grid (G); T1 (Test 1, Retrieval/Reactivation Test); T2-Tn (Test 2 to Test n, Post-reconsolidation Tests)

processes required for reconsolidation. Consistent with the reconsolidation hypothesis, behaviors linked to drug-induced memories have been disrupted by various post-retrieval treatments, including protein synthesis inhibitors, NMDA antagonists, noradrenergic receptor antagonists and zif 268 antisense oligonucleotide treatment (Bernardi et al., 2006; Fricks-Gleason and Marshall, 2008; Lee and Everitt, 2008; Robinson and Franklin, 2007a;Von der Goltz et al., 2009).

Disruption of reconsolidation is only one possible explanation of learned behavior deficits produced by post-retrieval manipulations. For example, a post-retrieval decrease in performance might be due simply to a transitory impairment of retrieval, not a permanent loss of memory (Lattal and Abel, 2004; Vianna et al., 2001). Furthermore, because a memory retrieval test involving US omission can also serve as an extinction trial that reduces the ability of the CS to evoke the original conditioned response by establishing a new CS→no-US association (Bouton, 1993; Pavlov, 1927; Rescorla, 2001), post-retrieval treatments might facilitate later performance deficits by altering the neurotransmitter or molecular mechanisms normally involved in extinction learning (Stafford and Lattal, 2011). Importantly, performance deficits are not the only possible outcome of post-retrieval manipulations. For example, post-retrieval treatments might maintain or enhance performance of learned behaviors by promoting transcriptional events (Stafford and Lattal, 2011) or by preventing the synaptic modifications necessary to consolidate new extinction memories (Berlau and McGaugh, 2006; Power et al., 2006). Thus, post-retrieval manipulations can either impair or enhance later performance of previously learned behaviors through several different hypothesized mechanisms.

It is well established that the noradrenergic system is involved in both memory consolidation and reconsolidation, especially for emotionally arousing events (Bernardi et al.,

2006; Diergaarde et al., 2006; McGaugh, 2000; Sara, 2009). For example, consolidation of fear, spatial and taste memories (Cahill et al., 2000; Grillon et al., 2004; Miranda et al., 2003) as well as odor-reward associations and inhibitory avoidance (Lennartz et al., 1996; Wilson et al., 1994) can be blocked by administration of noradrenergic receptor antagonists. Evidence implicating the noradrenergic system in the reconsolidation of fear (Rodriguez-Romaguera et al., 2009), spatial (Przybyslawski et al., 1999) and appetitive memories has also been reported (Diergaarde et al., 2006).

Recent evidence suggests that a single post-retrieval exposure to propranolol (PRO), a nonspecific ß-adrenergic receptor (ß-AR) antagonist, can impair subsequent performance of both cocaine- (Bernardi et al., 2006; Fricks-Gleason and Marshall, 2008) and morphine-induced CPP (Robinson and Franklin, 2007a; 2010), potentially implicating the noradrenergic system in the reconsolidation of drug-seeking behavior. In contrast, other studies found no effect of a single post-retrieval PRO injection on the reconsolidation of ethanol-induced memories as indexed by cue-induced reinstatement by ethanol self-administration in rats (Williams and Harding, 2011; Wouda et al., 2010). Repeated post-retrieval PRO injections reduced reinstatement in one of those studies (Wouda et al., 2010), but not in the other (Williams and Harding, 2011). In a study of pre-retrieval treatment effects on reconsolidation in rats, PRO also had no effect on subsequent responding to ethanol-associated cues in either pavlovian conditioned approach or pavlovian-instrumental transfer procedures (Milton et al., 2011). However, no studies have tested whether systemic PRO treatment would affect reconsolidation of ethanol-induced CPP. Understanding the role of the noradrenergic system in ethanol-induced conditioned memories will allow us to investigate whether ethanol shares mechanisms with other drugs of abuse. Addressing the generality of the PRO effect is important because, as previously proposed, the

molecular mechanisms underlying drug-induced CPP might not be the same for all abused drugs (Groblewski et al., 2011; Kuo et al., 2007).

The present studies examined the effects of post-retrieval PRO treatment on reconsolidation of ethanol-induced CPP. It has previously been proposed that memory strength affects sensitivity to reconsolidation (Robinson and Franklin, 2010; Suzuki et al., 2004). Thus, in order to more thoroughly evaluate the sensitivity of ethanol-induced CPP to PRO effects on reconsolidation, we varied our conditioning parameters across experiments (by varying the ethanol conditioning dose and the number of conditioning trials) in order to assess post-retrieval PRO effects on reconsolidation of a weak (Experiment 1), moderate (Experiment 2) and strong (Experiment 3) ethanol-induced CPP. Experiment 3 also examined the effect of repeated post-test PRO exposure on CPP extinction.

2. Materials and Methods

2.1. Subjects

DBA/2J male mice, 6 to 7 weeks old upon arrival to the laboratory, were purchased from Jackson Laboratory (Sacramento, CA). Mice were housed in groups of four per cage on a Thoren rack with standard laboratory rodent chow and tap water available *ad libitum*. They were maintained in the colony room for 2 weeks prior to experimentation at 21±1°C on a 12 h light/dark cycle (lights on at 7:00 a.m.). Experiments were performed during the light portion of the cycle beginning at 10:00 a.m. Protocols were approved by the OHSU Institutional Animal Use and Care Committee and were conducted in accordance with National Institutes of Health *Principles of Laboratory Animal Care*.

2.2. Apparatus

The apparatus consisted of 12 identical chambers (30 cm long x 15 cm wide x 20 cm high) contained in individual sound- and light-attenuated enclosures (Model E10-20; Coulbourn Instruments, Allentown, PA). Each conditioning box was equipped with six, equally spaced, infrared emitter/detector pairs running the length of the box, 2.2 cm above the floor and 5 cm apart. These detectors provided activity counts (expressed as beam breaks per min) and side preference (expressed as time spent on the left and right sides) during all conditioning and test sessions. Activity and side preference data were collected using a computer. No divider was used in the place conditioning chambers so animals had access to the entire box. For a detailed description of the apparatus see Cunningham et al. (2006). Tactile cues (interchangeable grid and hole floors) were used as the CSs. The grid floor consisted of 2.3 mm stainless steel rods mounted 6.4 mm apart on acrylic rails. Perforated stainless steel sheet metal (16 gauge) containing 6.4 mm round holes on 9.5 mm staggered centers was used as the hole floor. This combination of floor textures was selected based on previous studies showing that drug-naive control mice show no preference for either floor type (Cunningham et al., 2003).

2.3. Drugs

Ethanol solutions (20% v/v in physiological saline) were prepared from 95% ethanol and administered intraperitoneally (IP) at doses of 1 and 2 g/kg (6.25 and 12.5 ml/kg, respectively). These doses were selected based on previous studies of ethanol-induced CPP in DBA/2J mice (e.g., Groblewski et al., 2008, 2009). Dl-Propranolol (PRO) was obtained from Sigma Aldrich (St Louis, MO). PRO was dissolved in warmed isotonic saline (10 and 30 mg/ml) and injected IP at doses of 10 and 30 mg/kg (10 ml/kg). Doses and injection times were based on previous studies demonstrating efficacy in disrupting reconsolidation of CPP memories (Bernardi et al.,

2006; Fricks-Gleason and Marshall, 2008). A previous study reported that the elimination half-life of PRO in whole brain and blood of rats is approximately 52-60 min after a systemic (i.v.) administration (Elghozi et al., 1979). Fresh solutions of PRO were prepared daily.

2.4. Behavioral procedures

Each experiment consisted of the following experimental phases: habituation, conditioning, retrieval (T1; first CPP reactivation test after conditioning) and reconsolidation (T2; CPP test given after the retrieval test) tests. To assess PRO effects on extinction of CPP, 19 tests were conducted on consecutive days in Experiment 3 (T1-T19; retrieval [T1] and reconsolidation [T2] tests also served as extinction trials).

2.4.1. Experiment 1: Effect of a single post-retrieval PRO treatment on the reconsolidation of a weak ethanol-induced CPP (two conditioning trials, low ethanol dose). Experiment 1 examined the effects of post-test (retrieval test) administration of two doses of PRO or vehicle on a subsequent test (reconsolidation test) of a relatively weak ethanol-induced CPP. On the basis of previous findings in our lab (Cunningham et al., 2002; Groblewski et al., 2008; 2009), we exposed mice to a procedure that involved fewer conditioning trials (two) and a lower ethanol dose (1 g/kg) than we typically use for ethanol-induced CPP (four trials, 2 g/kg). Mice (n = 72) were randomly divided into three groups (saline, 10 and 30 mg/kg). All groups received habituation, conditioning, reactivation and reconsolidation tests. During habituation, mice received an IP injection of saline and were immediately placed inside the conditioning box on a smooth paper floor (without the conditioned stimuli) for 5-min. The conditioning phase (4 days) started the next day. During this phase, mice had 5-min access to the entire apparatus, but only one floor type was presented throughout the chamber. An unbiased CPP procedure was used in which mice were randomly assigned to one of two conditions (Grid+ [G+] or Grid- [G-])

(Cunningham et al., 2003). Mice in each PRO dose group were randomly assigned to one of two conditioning subgroups (n = 12/subgroup). Animals in the G+ group received an injection of ethanol (1 g/kg) paired with the grid floor (CS+) and an injection of saline paired with the hole floor (CS-). Alternatively, animals in the G- group received saline paired with the grid floor (CS-) and ethanol paired with the hole floor (CS+). Exposure to each floor CS, as well as type of injection (i.e., ethanol vs. saline), alternated across conditioning sessions in a counterbalanced manner.

Forty-eight hr after the last conditioning session, mice were injected with saline and placed in the center of the conditioning chamber prepared with both grid and hole floors for a memory reactivation test (T1). Position of each floor type was counterbalanced within each conditioning subgroup. Activity and side preference were monitored for 15 min. Immediately after the test, mice were injected with PRO (0, 10 or 30 mg/kg) and returned to the home cage. This timing was chosen based on previous studies demonstrating a disruptive effect of PRO on reconsolidation of cocaine CPP after a 15-min memory retrieval test (Bernardi et al., 2006). Twenty-four hr later, mice received a second 15-min preference test to assess the effects of post-retrieval PRO exposure (reconsolidation test, T2). Because no significant disruption of memory was seen on the reconsolidation test, three additional 15-min tests were conducted on each of the next 3 days (T3-T5). Post-retrieval injections were not given after these tests. The purpose of these tests was to see whether the effect of the PRO injection given after the first retrieval test might be revealed during repeated testing.

2.4.2. Experiment 2: Effect of a single post-retrieval PRO treatment on the reconsolidation of a moderate ethanol-induced CPP (two conditioning trials, high ethanol dose). The purpose of this study was to evaluate the effect of a single post-retrieval PRO injection on

the reconsolidation of an ethanol conditioned memory that was somewhat stronger than that established in Experiment 1. In order to increase CPP strength, mice were injected with an ethanol dose of 2 g/kg (instead of 1 g/kg) on both of the CS+ conditioning trials. Otherwise, the experimental design and procedures were identical to those used in Experiment 1.

2.4.3. Experiments 3A and 3B: Effect of a single and repeated post-retrieval PRO treatment on the reconsolidation and extinction of a strong ethanol-induced CPP (four trials, high dose). The first aim of these experiments was to investigate the effect of PRO given immediately after reactivation on reconsolidation of a strong ethanol conditioned memory (Experiments 3A and 3B). The second aim was to assess the effect of repeated immediate post-test PRO injections on extinction of ethanol-induced CPP (Experiment 3B). Mice in both experiments (n = 48/experiment) were randomly assigned to either G+ or G- conditioning subgroups within each of two PRO treatment groups (saline or PRO 10 mg/kg). Mice were then exposed to habituation and conditioning sessions identical to those used for Experiment 2, except that conditioning continued until all mice had received a total of four CS+ and four CS- conditioning trials. This combination of dose (2 g/kg) and number of trials (four) has previously been reported to produce an asymptotic level of ethanol-induced CPP in DBA2/J mice (Groblewski et al., 2008). The first retrieval/reactivation test (T1) occurred 24 h after the last conditioning trial and was immediately followed by a post-retrieval injection of saline or PRO. As in earlier experiments, a reconsolidation test (T2) was given on the next day.

In contrast to the previous studies, mice in Experiment 3B also received saline or PRO injections after the reconsolidation test and they continued to receive injections after each of the next 17 tests (conducted on consecutive days) in order to determine whether repeated post-test exposure to PRO would alter the course of CPP extinction. In order to control for possible non-

specific effects of repeated exposure to PRO, mice in the saline control group received a PRO injection 3 h after each test in their home cages (mice in the PRO group received a home cage injection of saline at the same time). The rationale for this delay was based on the idea that drugs that modulate memory consolidation should be less effective many hours after memory retrieval (McGaugh and Roozendaal, 2009; Przybyslawski et al., 1999).

2.5. Data analyses

The primary dependent variable in these studies was the amount of time spent on the grid floor (grid times) during the memory retrieval, reconsolidation, extinction, and postreconditioning preference tests. In this unbiased counterbalanced CPP procedure, differences between the G+ and G- conditioning subgroups in time spent on the grid floor are used to index strength of place conditioning (Cunningham et al., 2003; 2006). However, to simplify presentation of the time-course of extinction across repeated preference tests, we also converted these data to percent time spent on the drug-paired floor (collapsed across conditioning subgroups). Test data were analyzed by means of one-way, two-way or three-way ANOVAs with repeated measures (treatment x conditioning subgroup x test). Treatment (0, 10 and 30 mg⁄kg of PRO) and conditioning subgroup (G+ vs. G-) were treated as between group factors, whereas test was treated as a within-group factor. Alpha-level was set at 0.05 for all analyses.

3. Results

3.1. Experiment 1: Effect of a single post-retrieval PRO treatment on the reconsolidation of a weak ethanol-induced CPP (two conditioning trials, low ethanol dose). As noted earlier, the strength of the memory may affect the susceptibility to disrupt memory during reconsolidation (Robinson and Franklin, 2010; Suzuki et al., 2004). Thus, Experiment 1 was designed to examine the effect of a single post-retrieval injection of PRO on the reconsolidation of a weak

ethanol-induced CPP. As can be seen in Figure 1a, mice expressed a weak but reliable place preference during the post-conditioning retrieval test (T1). That is, mice that had previously received pairings of ethanol with the grid floor (G+ subgroups) consistently spent more time on the grid floor during testing than mice that had previously received saline paired with the grid floor (G- subgroups). However, post-test administration of PRO failed to produce a significant reduction in CPP during the reconsolidation test 24 h later (T2, Figure 1b), indicating that PRO did not affect the reconsolidation of a weak ethanol-induced CPP.

A three-way (treatment x conditioning subgroup x test) repeated measures ANOVA supported these conclusions, showing a significant main effect of conditioning subgroup [$F_{1,66} = 19.4$, $p < 0.01$], but no effect of treatment group or three-way interaction. The overall analysis also showed a significant treatment x test interaction [$F_{2,66} = 3.6$, $p < 0.05$], suggesting that PRO might have produced a non-associative shift in overall preference for the grid floor in one of the groups after the first CPP test. Follow-up two-way ANOVAs (conditioning subgroup x test) conducted separately for each group confirmed this interpretation, showing that there was a significant decrease in time spent on the grid floor by mice in the 30 mg/kg group regardless of conditioning subgroup [$F_{1,22} = 7.8$, $p < 0.05$], but no test effect in either the saline or 10 mg/kg groups. Nevertheless, in all three follow-up analyses, there was a significant main effect of conditioning subgroup [all p's < 0.05] and no interaction, showing that PRO had no selective effect on CPP.

Insert Figure 1 about here

To address the possibility that effects of post-retrieval PRO might be revealed with repeated testing, we conducted three additional tests on subsequent days with no further exposure to PRO. Each of these tests was identical to the initial reconsolidation test. To simplify presentation and analysis, Figure 1c depicts performance during all five post-conditioning tests as percent time spent on the ethanol-paired floor. Two-way repeated measures ANOVA (treatment x test) revealed no effect of PRO treatment, no effect of test and no interaction. Thus, the results showed that PRO did not affect retrieval of ethanol-induced CPP even when we tested for several days after the presumed reconsolidation of a labile conditioned memory.

3.2. Experiment 2: Effect of a single post-retrieval PRO treatment on the reconsolidation of a moderate ethanol-induced CPP (two conditioning trials, high ethanol dose). The purpose of this experiment was to examine the effect of PRO on reconsolidation of a moderate strength CPP induced by using a higher ethanol dose (2 g/kg) than that used in Experiment 1. Other than the dose, the design and procedure were identical to those in the previous experiment. Figure 2a shows performance for all groups during the first post-conditioning retrieval test (T1), whereas Figure 2b shows the effect of post-retrieval PRO on reconsolidation test performance (T2). As in Experiment 1, PRO did not interfere with reconsolidation at either dose. In fact, preference (indexed by the difference between the G+ and G- conditioning subgroups) was somewhat stronger during the reconsolidation test (T2) than during the retrieval test (T1). These conclusions were supported by a three-way repeated measures ANOVA (treatment x conditioning subgroup x test) that revealed a significant main effect of conditioning subgroup [$F_{1,64} = 98.2$, $p < 0.01$], but no effect of post-retrieval treatment (0, 10 or 30 mg/kg) or three-way interaction. The overall ANOVA also yielded a conditioning subgroup x test interaction [$F_{1,64} = 8.6$, $p < 0.01$], reflecting the general increase in preference for the ethanol paired floor after the

first preference test (T1). However, post-retrieval PRO had no impact on this increase as the 3-way interaction was not significant. Moreover, in contrast to Experiment 1, post-retrieval exposure to the high PRO dose did not produce a non-associative shift in time spent on the grid floor during the reconsolidation test (T2).

Insert Figure 2 about here

As in Experiment 1, we also evaluated the effect of the post-retrieval PRO injection across several repeated tests with no further exposure to PRO. Performance during all five post-conditioning tests (T1-T5) is depicted in Figure 2c as percent time spent on the ethanol-paired floor. As can be seen, all treatment groups continued to express a similar magnitude CPP over the course of four post-retrieval tests, indicating that there was no delayed effect of the single post-retrieval PRO injection on later performance. Two-way repeated measures ANOVA (treatment x test) supported this conclusion, yielding only a significant main effect of test [$F_{4,268} = 6.5$, $p < 0.01$], but no treatment effect or interaction. Two mice were removed from the study, one because of a procedural error during testing (saline group) and the other because of a health problem during conditioning (PRO 30 mg/kg group).

3.3. Experiments 3A and 3B: Effect of a single and repeated post-retrieval PRO treatment on the reconsolidation and extinction of a strong ethanol-induced CPP (four trials, high dose). Following the same rationale used for our previous studies, Experiments 3A and 3B examined the effects of post-retrieval administration of PRO (10 mg/kg) on a subsequent test of a strong ethanol-induced CPP. The conditioning parameters were identical to those shown in previous studies to produce an asymptotic level of ethanol-induced CPP (e.g., Groblewski et al.,

2008). Mice in both experiments expressed a robust and similar CPP both before (Figures 3a and 3c) and after (Figures 3b and 3d) PRO treatment, indicating that post-retrieval PRO treatment did not disrupt the reconsolidation of a strong ethanol-induced CPP. Supporting this conclusion, a three-way repeated measures ANOVA (treatment x conditioning subgroup x test) revealed a significant main effect of conditioning subgroup for Experiments 3A [$F_{1,43} = 58.2$, $p < 0.01$] and 3B [$F_{1,44} = 64.74$, $p < 0.01$], but no treatment effect or interactions. In contrast to Experiment 2, there was no increase in CPP magnitude after the retrieval test. One mouse from the saline group was removed from all statistical analyses in Experiment 3A due to a procedural error during conditioning.

Insert Figure 3 about here

Given the consistent lack of a PRO effect on reconsolidation of ethanol-induced CPP across all three experiments, we decided to assess the effects of repeated post-retrieval PRO treatment on extinction of ethanol-induced CPP (Experiment 3B only). Performance during all 19 post-conditioning tests is depicted in Figure 4 as percent time spent on the ethanol-paired floor. As can be seen, both saline and PRO groups expressed a persistent preference for the ethanol-paired compartment that decreased across all 19 post-conditioning tests. However, there was no difference between treatment groups. This conclusion was supported by a two-way repeated measures ANOVA (treatment x test) that showed a significant main effect of test [$F_{18,828} = 6.09$, $p < 0.01$] but no significant interaction. Follow-up repeated measures ANOVAs comparing preference for the grid floor on T1 *vs* T19 (data not shown) confirmed that there was main effect of conditioning subgroup [$F_{1,44} = 38.22$, $p < 0.01$], and a test by conditioning subgroup interaction [$F_{1,44} = 23.31$, $p < 0.01$], indicating a decrease in preference over time. These results are generally in agreement with previous data showing that repeated testing of a

strong ethanol-induced CPP produces extinction in vehicle treated mice (Groblewski et al., 2009).

Insert Figure 4 about here

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4. Discussion

Contrary to the prediction of the reconsolidation hypothesis, our results showed that post-retrieval administration of PRO, a nonspecific ß-AR antagonist, did not modify subsequent performance of ethanol-induced CPP, suggesting there was no change in memory retention. This outcome was observed using two different PRO doses (10 and 30 mg/kg) across three separate experiments that manipulated CPP strength by varying the number of conditioning trials (2 or 4) or the ethanol-conditioning dose (1 or 2 g/kg). Our last experiment also showed that repeated administration of PRO after consecutive retrieval tests had no effect on extinction of ethanol-induced CPP in mice. This result was observed after four ethanol conditioning trials, suggesting that the ß-adrenergic receptor does not modulate performance during the extinction of a well-established conditioned effect of ethanol. Altogether, our data suggest that the noradrenergic system is involved in neither the reconsolidation nor the extinction of ethanol-conditioned memories in mice under our experimental conditions.

4.1 Reconsolidation of ethanol related memories

The lack of a PRO effect on reconsolidation of ethanol-induced CPP contrasts with previous studies demonstrating a role for the noradrenergic system in the reconsolidation of both cocaine- (Bernardi et al., 2006; Bernardi et al., 2009; Fricks-Gleason and Marshall, 2008) and morphine-induced CPP (Robinson and Franklin, 2007a; 2010). Since we used PRO at the same and higher doses than those used in earlier studies, the discrepant outcome is not readily

explained by inadequate dose selection. Also, because we systematically varied the strength of CPP across experiments, the absence of a PRO effect cannot be explained by arguing that the memory was too strong (or too weak) to be disrupted by a post-retrieval manipulation. Moreover, the fact that repeated post-treatment tests without additional PRO exposure failed to reveal any delayed effect of the initial PRO treatment argues against the suggestion that there was insufficient opportunity for such effects to be detected (Experiments 1-2).

Previous studies from other laboratories have generally reported no effect of a single exposure to the ß-AR antagonist PRO on the reconsolidation of ethanol-related memories in instrumental (operant) self-administration procedures (Milton et al., 2011; Williams and Harding, 2011; Wouda et al., 2010). Also, no effect of PRO was seen on reconsolidation of an ethanol memory in a pavlovian conditioned approach procedure (Milton et al., 2011). Interestingly, repeated PRO exposure in consecutive tests was reported to gradually reduce cue-induced reinstatement after extinction of ethanol self-administration, suggesting either a cumulative effect of disrupting reconsolidation or facilitation of extinction (Wouda et al., 2010). However, this effect was not replicated in another study of PRO's effect on cue-induced reinstatement in a similar procedure (Williams and Harding, 2011). Although drug-induced reinforcement can be measured by both pavlovian and instrumental procedures, several studies indicate a clear neuropharmacological and neuroanatomical dissociation between them (for a review see Bardo and Bevins, 2000). Thus, discrepancies between studies involving pavlovian vs. instrumental procedures might be attributable to differences in the neurotransmitter systems required for reconsolidation of the memories underlying ethanol-induced CPP, conditioned approach and self-administration.

Also, aside from being functionally different from an instrumental procedure, a pavlovian

procedure might also have different consequences in terms of memory retrieval. For example, Riccio et al. (2006) suggested that the degree of reactivation and the nature of the memory attributes reactivated could be different when using different procedures. Therefore, we cannot dismiss the possibility that other tasks would make ethanol-conditioned memories more vulnerable to the amnesic effects of PRO after a retrieval test.

Another possibility that must be considered is that PRO's ability to disrupt reconsolidation of drug-induced memories might depend on the specific neurotransmitter pathways that are activated by the unconditioned stimulus drug or by contextual cues previously associated with that drug. Also, we cannot exclude the possibility that intracerebral administration of PRO into specific brain areas might affect ethanol reconsolidation. Although the ß-adrenergic receptor appears to mediate the reconsolidation of cocaine- (Bernardi et al., 2006) and morphine- (Robinson and Franklin, 2007a) induced CPP, it is possible that the activation of ethanol-conditioned memories is independent of the molecular pathways that are activated through noradrenaline receptors. General support for this argument has been provided by previous studies suggesting that the molecular mechanisms underlying drug-induced CPP may not be the same for all the abused drugs (e.g., Groblewski et al., 2011; Kuo et al., 2007). Future studies are necessary to determine the specific neuropharmacological systems and brain areas that are involved in the maintenance of ethanol-induced conditioned memories.

An important issue that was not addressed in these or previous studies is whether PRO affects the initial consolidation of ethanol-induced CPP. If the ß-AR were not involved in the consolidation of this type of ethanol-induced learning, its non-involvement in the reconsolidation and extinction of ethanol-induced CPP might be less surprising. Interestingly, there do not appear to be any studies that have examined the effect of PRO on the initial consolidation of CPP

induced by any other abused drug, so it is not known whether previously reported effects of PRO on reconsolidation of cocaine- and morphine-induced CPP are mirrored by similar effects on the initial consolidation of CPP induced by these drugs. Although strong evidence has been established for an influence of noradrenaline on memory consolidation, it is possible that the activation of ß-AR is not required for some types of learning, including the acquisition and extinction of ethanol-induced CPP. Future research must resolve these issues.

4.2 Extinction of ethanol related memories

The mechanism underlying extinction of conditioned memories involves a new form of inhibitory learning in which the CS progressively loses its ability to evoke the conditioned response (Bouton, 1993; Pavlov, 1927; Rescorla, 2001). Noradrenaline efflux is increased in the medial prefrontal cortex upon extinction of an appetitive conditioned stimulus, suggesting the involvement of this system in extinction learning (Mingote et al., 2005). It has been proposed that noradrenaline induces the synaptic modifications necessary to consolidate new memories via ß-AR activation, including memories unique to extinction (Berlau and McGaugh, 2006; Mueller and Cahill, 2010; Sara, 2009). Hence, by blocking the ß-AR, PRO should prevent extinction of drug-conditioned memories. However, our results revealed no effect on the extinction rate in the animals treated with PRO immediately after 18 consecutive extinction tests. That is, groups receiving either an immediate post-retrieval injection of PRO or saline both extinguished similarly.

Our findings are in agreement with previous research showing no effect of PRO treatment on extinction of fear conditioning (Rodriguez-Romaguera et al., 2009) or cue-induced reinstatement of ethanol self-administration (Williams and Harding, 2011), but they are not consistent with other studies suggesting that PRO facilitated extinction of cue-induced

reinstatement of ethanol self-administration (Wouda et al., 2010) and cocaine-induced CPP (Fricks-Gleason & Marshall, 2008; Otis & Mueller, 2011). Conflicting outcomes have also been reported in extinction studies involving non-drug reinforcers. For example, PRO administration has sometimes disrupted (Cohen and Gotthard, 2011; Terry et al., 1990), facilitated (Terry et al., 1990) or had no effect on extinction in appetitive (food) learning tasks (Terry et al., 1990), suggesting that the processes underlying retention of those memories were different.

Procedural differences across studies could explain conflicting results observed after PRO administration. For example, different effects have been reported depending on the timing of PRO administration relative to reactivation of the memory after conditioning. Otis and Mueller (2011) found that PRO injection 20 min before the first choice test interfered with the expression of cocaine-induced CPP on that test as well as during a second drug-free choice test 24 h later. However, injection of PRO immediately before the first test did not affect CPP expressed on either test. The first outcome was interpreted as a PRO-induced permanent impairment in memory retrieval whereas the second outcome was explained as a failure of PRO to affect reconsolidation of the cocaine memory, an interpretation that conflicts with previous studies showing a disruptive effect of post-retrieval PRO exposure on reconsolidation of cocaine-induced CPP (Bernardi et al., 2006). Differences between effects of pre- versus post-retrieval PRO injection on reconsolidation have also been reported in an aversive task (Puig et al., 2009).

Another explanation for the lack of a PRO effect on extinction might be that ß-AR participation is related to parameters of CS re-exposure that affect rate of extinction. As can be seen in Figure 4, ethanol-induced CPP in our last experiment was relatively resistant to extinction, requiring 19 consecutive days of 15-min exposures to the test apparatus. Although our earlier experiments showed that strength of conditioning, per se, is unlikely to explain PRO's

failure to affect reconsolidation, PRO's ability to influence extinction might have been more apparent under conditions that produced more rapid extinction (e.g., 30-min, instead of 15-min extinction sessions—see Groblewski et al., 2009). In other words, ß-AR might play a more important role under conditions that normally facilitate extinction, without being critically involved when tested in procedures that show a profile more resistant to extinction.

It is interesting to note that Janak and Corbit (2011) recently found that PRO impaired extinction of the response to an appetitive discriminative stimulus following presentation of a compound (light + sound), but not after presentation of a simple stimulus (sound) during extinction. Thus, other procedural differences such as the nature of the stimuli used in the studies may explain the discrepancies between our results and previous findings. Our CPP procedure involved the presentation of a single tactile cue whereas other studies demonstrating a role for the noradrenergic system in the reconsolidation or extinction of drug-induced CPP involved the presentation of a compound stimulus. For example, CPP chambers from Robinson and Franklin's (2007a; 2007b; 2010) morphine studies applied tactile and visual cues and in the Fricks-Gleason and Marshall's (2008) cocaine study the compartments were distinguished by different visual, olfactory, and tactile cues. In our studies, it is possible that the amount of attention and arousal engaged during extinction were sufficient to process the emotional valence of the tactile cues but not to recruit the noradrenergic system. If that were the case, PRO treatment after memory reactivation with a simple tactile cue should not have an effect on the extinction of alcohol related memories. On the basis of our findings, we posit that the activation of ß-AR is not necessary for the consolidation of alcohol extinction memory in the CPP procedure.

5. Conclusions

Overall, our data indicate that systemic post-retrieval administration of PRO, a non-selective ß-AR antagonist, failed to disrupt reconsolidation of ethanol-induced conditioned memories. Additionally, repeated post-retrieval exposure to PRO did not interfere with extinction of ethanol-CPP, suggesting no role of ß-ARs in the consolidation of extinction. On the basis of previous preclinical findings, there has been increasing interest in the use of ß-blockers (including PRO) to prevent drug-induced seeking behavior. If memories can be disrupted with ß-blockers during memory reactivation, drugs such as PRO could be effective in the treatment of drug addiction (Taylor et al., 2009; Tronson and Taylor, 2007). However, our data suggest that reconsolidation and extinction of ethanol-seeking behavior might be independent of ß-AR activation. Under our experimental conditions, other neurotransmitter systems may be responsible for maintenance of ethanol-induced conditioned effects. Additional studies, including studies involving alternative procedures, are needed to more completely address the role of the noradrenergic system in the acquisition, consolidation, retrieval, reconsolidation and extinction of ethanol-related memories.

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7. References

Alberini CM, Milekic MH, Tronel S. Mechanisms of memory stabilization and de-stabilization. Cell Mol Life Sci 2006;63:999-1008.

Bardo MT, Bevins RA. Conditioned place preference: what does it add to our preclinical understanding of drug reward? Psychopharmacology 2000;153:31-43.

Berlau DJ, McGaugh J. Enhancement of extinction memory consolidation: the role of the noradrenergic and GABAergic systems within the basolateral amygdala. Neurobiol Learn Mem 2006;86:123-32.

Bernardi RE, Lattal KM, Berger SP. Postretrieval propranolol disrupts a cocaine conditioned place preference. Neuroreport 2006;17:1443-47.

Bernardi RE, Ryabinin, AE, Berger SP, Lattal KM. Post-retrieval disruption of a cocaine conditioned place preference by systemic and intrabasolateral amygdala beta2- and alpha1-adrenergic antagonists. Learn Mem 2009;16:777-89.

Bouton ME. Context, time, and memory retrieval in the interference paradigms of Pavlovian learning. Psychol Bull 1993;114:80-99.

Cahill L, Pham CA, Setlow B. Impaired memory consolidation in rats produced with beta-adrenergic blockade. Neurobiol Learn Mem 2000;74:259-66.

Cohen J, Gotthard GH. Extinction of appetitive learning is disrupted by cycloheximide and propranolol in the sand maze in rats. Neurobiol Learn Mem 2011;95:484-90.

Cunningham CL, Ferree NK, Howard MA. Apparatus bias and place conditioning with ethanol in mice. Psychopharmacology 2003;170:409-22.

Cunningham CL, Gremel CM, Groblewski PA. Drug-induced conditioned place preference and aversion in mice. Nat Protoc 2006;1:1662-70.

Cunningham CL, Tull LE, Rindal KE, Meyer PJ. Distal and proximal pre-exposure to ethanol in the place conditioning task: tolerance to aversive effect, sensitization to activating effect, but no change in rewarding effect. Psychopharmacology 2002;160:414-24.

Davis HP, Squire LR. Protein synthesis and memory: a review. Psychol Bull 1984;96:518-59.

Diergaarde L, Schoffelmeer AN, De Vries TJ. Beta-adrenoceptor mediated inhibition of long-term reward-related memory reconsolidation. Behav Brain Res 2006;170:333-36.

Dudai Y. The neurobiology of consolidations, or, how stable is the engram?. Annu Rev Psychol 2004;55:51-86.

Dudai Y. The shaky trace. Nature 2000;406:686-87.

Elghozi JL, Bianchetti G, Morselli PL, Meyer P. Brain distribution of propranolol in the rat. Eur J Pharmacol. 1979; 55:319-22.

Fricks-Gleason AN, Marshall JF. Post-retrieval beta-adrenergic receptor blockade: effects on extinction and reconsolidation of cocaine-cue memories. Learn Mem 2008;15:643-48.

Grillon C, Cordova J, Morgan CA, Charney DS, Davis M. Effects of the beta-blocker propranolol on cued and contextual fear conditioning in humans. Psychopharmacology 2004;175:342-52.

Groblewski PA, Bax LS, Cunningham CL. Reference-dose place conditioning with ethanol in mice: empirical and theoretical analysis. Psychopharmacology 2008;201:97-106.

Groblewski PA, Franken FH, Cunningham CL. Inhibition of extracelular signal-regulated kinase (ERK) activity with SL327 does not prevent acquisition, expression, and extinction of ethanol-seeking behavior in mice. Behav Brain Res 2011;217:399-407.

Groblewski PA, Lattal KM, Cunningham CL. Effects of D-cycloserine on extinction and reconditioning of ethanol-seeking behavior in mice. Alcohol Clin Exp Res 2009;33:772-82.

Janak PH, Corbit LH. Deepened extinction following compound stimulus presentation: noradrenergic modulation. Learn Mem 2011;18:1-10.

Kuo YM, Liang KC, Chen HH, Cherng CG, Lee HT, Lin Y, Huang AM, Liao RM, Yu L. Cocaine-but not methamphetamine-associated memory requires de novo protein synthesis. Neurobiol Learn Mem 2007;87:93-100.

Lattal KM, Abel T. Behavioral impairments caused by injections of the protein synthesis inhibitor anisomycin after contextual retrieval reverse with time. 2004. Proc Natl Acad Sci USA 2004;101:4667-72.

Lee JL, Everitt BJ. Appetitive memory reconsolidation depends upon NMDA receptor-mediated neurotransmission. Neurobiol Learn Mem 2008;90:147-54.

Lennartz RC, Hellems KL, Mook ER, Gold PE. Inhibitory avoidance impairments induced by intra-amygdala propranolol are reversed by glutamate but not glucose. Behav Neurosci 1996;110:1033-39.

McGaugh JL, Roozendaal B. Drug enhancement of memory consolidation: historical perspective and neurobiological implications. Psychopharmacology 2009;202:3-14.

McGaugh JL. Memory: a century of consolidation. Science 2000;287:248-51.

Milton AL, Schramm MJ, Wawrzynski JR, Gore F, Oikonomou-Mpegeti F, Wang NQ, Samuel D, Economidou D, Everitt BJ. Antagonism at NMDA receptors, but not β-adrenergic receptors, disrupts the reconsolidation of pavlovian conditioned approach and instrumental transfer for ethanol-associated conditioned stimuli. Psychopharmacology 2011;DOI: 10.1007/s00213-011-2399-9

Mingote S, De Bruin JP, Feenstra MG. Noradrenaline and dopamine efflux in the prefrontal cortex in relation to appetitive classical conditioning. J Neurosci 2005;24:2475-80.

Miranda MI, LaLumiere RT, Buen TV, Bermudez-Rattoni F, McGaugh JL. Blockade of noradrenergic receptors in the basolateral amygdala impairs taste memory. Eur J Neurosci 2003;18:2605-10.

Mueller D, Cahill SP. Noradrenergic modulation of extinction learning and exposure therapy. Behav Brain Res 2010;208:1-11.

Nader K, Schafe GE, LeDoux JE. The labile nature of consolidation theory. Nat Rev Neurosci 2000;1:216-19.

Otis JM, Mueller D. Inhibition of β-Adrenergic Receptors Induces a Persistent Deficit in Retrieval of a Cocaine-Associated Memory Providing Protection against Reinstatement. Neuropsychopharmacology. 2011;36:1912-20.

Pavlov I. Conditioned reflexes. London: Oxford UP; 1927.

Pedreira ME, Pérez-Cuesta LM, Maldonado H. Reactivation and reconsolidation of long-term memory in the crab Chasmagnathus: protein synthesis requirement and mediation by NMDA-type glutamatergic receptors. J Neurosci 2002;22:8305-11.

Power AE, Berlau DJ, McGaugh JL, Steward O. Anisomycin infused into the hippocampus fails to block "reconsolidation" but impairs extinction: the role of re-exposure duration. Learn Mem 2006;13:27-34.

Przybyslawski J, Roullet P, Sara SJ. Attenuation of emotional and nonemotional memories after their reactivation: role of beta adrenergic receptors. J Neurosci 1999;19:6623-28.

Puig S, Oualian C and Gisquet-Verrier P. Propranolol rather interferes with memory reactivation than with memory reconsolidation. 41st European Brain and Behaviour Society Meeting. Front Behav Neurosci 2009;DOI: 10.3389/conf.neuro.08.2009.09.266

Rescorla RA. Retraining of extinguished Pavlovian stimuli. J Exp Psychol Anim Behav Process

2001;27:115-24.

Riccio DC, Millin PM, Bogart AR. Reconsolidation: a brief history, a retrieval view, and some recent issues. Learn Mem 2006;13:536-44.

Robinson MJ, Franklin KB. Central but not peripheral beta-adrenergic antagonism blocks reconsolidation for a morphine place preference. Behav Brain Res 2007a;182:129-34.

Robinson MJ, Franklin KB. Effects of anisomycin on consolidation and reconsolidation of a morphine-conditioned place preference. Behav Brain Res 2007b;178:146-53.

Robinson MJ, Franklin KB. Reconsolidation of a morphine place preference: Impact of the strength and age of memory on disruption by propranolol and midazolam. Behav Brain Res 2010;213:201-07.

Rodriguez-Romaguera J, Sotres-Bayon F, Mueller D, Quirk GJ. Systemic propranolol acts centrally to reduce conditioned fear in rats without impairing extinction. Biol Psychiatry 2009;65:887-92.

Sara SJ. Retrieval and reconsolidation: toward a neurobiology of remembering. Learn Mem 2000;7:73-84.

Sara SJ. The locus coeruleus and noradrenergic modulation of cognition. Nat Rev Neurosci 2009;10:211-23.

Stafford JM, Lattal KM. Is an epigenetic switch the key to persistent extinction?. Neurobiol Learn Mem 2011;96:35-40.

Suzuki A, Josselyn SA, Frankland PW, Masushige S, Silva AJ, Kida S. Memory reconsolidation and extinction have distinct temporal and biochemical signatures. J Neurosci 2004;24:4787-95.

Taylor JR, Olausson P, Quinn JJ, Torregrossa MM. Targeting extinction and reconsolidation

mechanisms to combat the impact of drug cues on addiction. Neuropharmacology 2009;56 Supp 1:186-95.

Terry P, Wray N, Salmon P. Acute and chronic effects of propranolol on extinction of rewarded running in the rat. Pharmacol Biochem Behav 1990;36:249-53.

Tronson, NC, Taylor JR. Molecular mechanisms of memory reconsolidation. Nat Rev Neurosci 2007;8:262-75.

Vianna MR, Szapiro G, McGaugh JL, Medina JH, Izquierdo I. Retrieval of memory for fear-motivated training initiates extinction requiring protein synthesis in the rat hippocampus. Proc Natl Acad Sci USA 2001;98:12251-54.

Von der Goltz C, Vengeliene V, Bilbao A, Perreau-Lenz S, Pawlak CR, Kiefer F, Spanagel R. Cue-induced alcohol-seeking behaviour is reduced by disrupting the reconsolidation of alcohol-related memories. Psychopharmacology 2009;205:389-97.

Williams KL, Harding KM The influence of adrenergic manipulation with yohimbine and propanolol on the effects of repeated ethanol cue-exposure session in rats. 34th Annual Scientific Meeting of the Research Society on Alcoholism June 25-29; Atlanta, Georgia. Alcohol Clin Exp Res 2011;35 Supp s1:1A–339A, PO695.

Wilson DA, Pham TC, Sullivan RM. Norepinephrine and posttraining memory consolidation in neonatal rats. Behav Neurosci 1994;108:1053-58.

Wouda JA, Diergaarde L, Riga D, van Mourik Y, Schoffelmeer AN, De Vries TJ. Disruption of Long-Term Alcohol-Related Memory Reconsolidation: Role of β-Adrenoceptors and NMDA Receptors. Front Behav Neurosci 2010;4:1-7.

8. Figure Captions

Figure 1. Post-retrieval PRO treatment did not affect reconsolidation of a weak ethanol-induced conditioned place preference (CPP) in mice. Mean ± SEM time spent on the grid floor for the grid+ (G+) and grid- (G-) subgroups during the 15-min drug-free test (n=72). Following 2 conditioning trials with 1 g/kg of ethanol all mice expressed weak but reliable place preference (Fig. 1a; T1=retrieval test; first CPP test after conditioning). However, post-retrieval PRO treatment (10 or 30 mg/kg) did not affect the reconsolidation of a weak ethanol-induced CPP (Fig. 1b; T2=reconsolidation test; CPP test given after the retrieval test). The results of 4 days of consecutive preference tests (T2-T5) after 1 single PRO injection are presented in Figure 1c (Mean ± SEM percent time spent for all groups on ethanol-paired floor). These results suggest that PRO did not modulate the retrieval of ethanol-induced CPP despite repeated testing over several days.

* Denotes a significant main effect of conditioning subgroup but no effect of treatment, $p < 0.01$).

Figure 2. Post-retrieval PRO treatment did not affect reconsolidation of a moderate ethanol-induced CPP in mice. Mean ± SEM time spent on the grid floor for the grid+ (G+) and grid- (G-) subgroups during the 15-min drug-free test (n=72). All groups showed preference following 2 ethanol (2 g/kg) conditioning trials (Fig. 2a; T1=retrieval test; first CPP test after conditioning). The effect of PRO after memory reactivation is depicted in Figure 2b (T2=reconsolidation test; CPP test given after the retrieval test). As is shown, PRO (10 or 30 mg/kg) did not block reconsolidation at either dose. This experiment also evaluated the effect of a single post-retrieval PRO injection during 4 subsequent preference tests. Performance on all 5

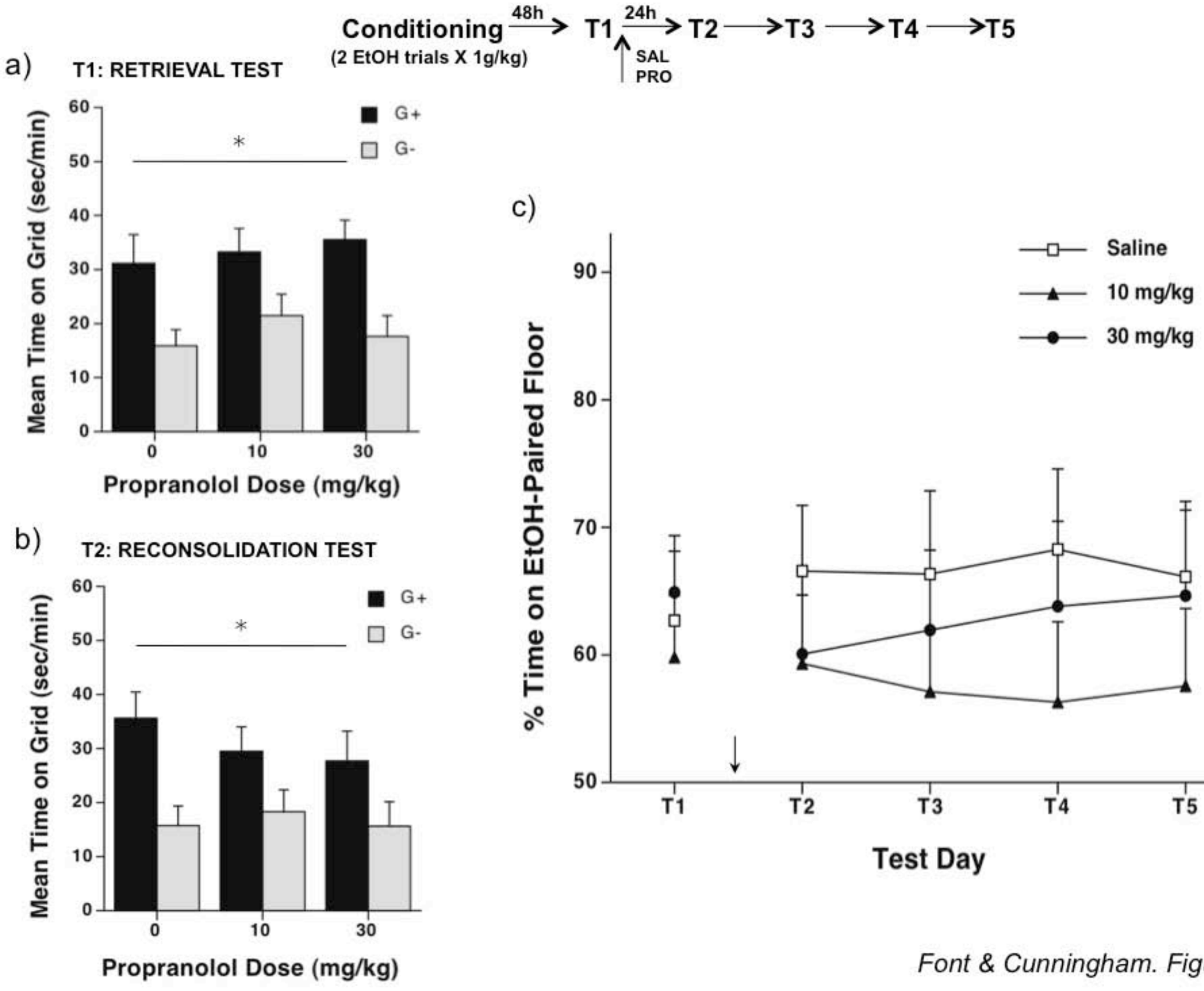
post-conditioning tests is depicted in Figure 2c (T2-T5) as mean ± SEM percent time spent on the ethanol-paired floor. As is shown, all groups (saline, 10 and 30 mg/kg) expressed conditioned preference over the course of 4 post-retrieval tests, after 1 single PRO injection. * Denotes a significant main effect of conditioning subgroup but no effect of treatment, $p < 0.01$).

Figure 3. Post-retrieval PRO treatment did not affect reconsolidation of a strong ethanol-induced CPP in mice. Data represent mean ± SEM time spent on the grid floor for the grid+ (G+) and grid- (G-) subgroups during the 15-min drug-free test (n=48). Both groups expressed a robust CPP after 4 conditioned trials with 2 g/kg of ethanol (Fig. 3a and Fig. 3c). Post-retrieval treatment of PRO (10 mg/kg) immediately after the first CPP test after conditioning (T1=retrieval test) did not disrupt the reconsolidation of ethanol-induced CPP (Fig. 3b and 3d; T2=reconsolidation test; CPP test given after the retrieval test). (HC): Refers to injections (saline or PRO) administered 3 h after T1 in their home cages.

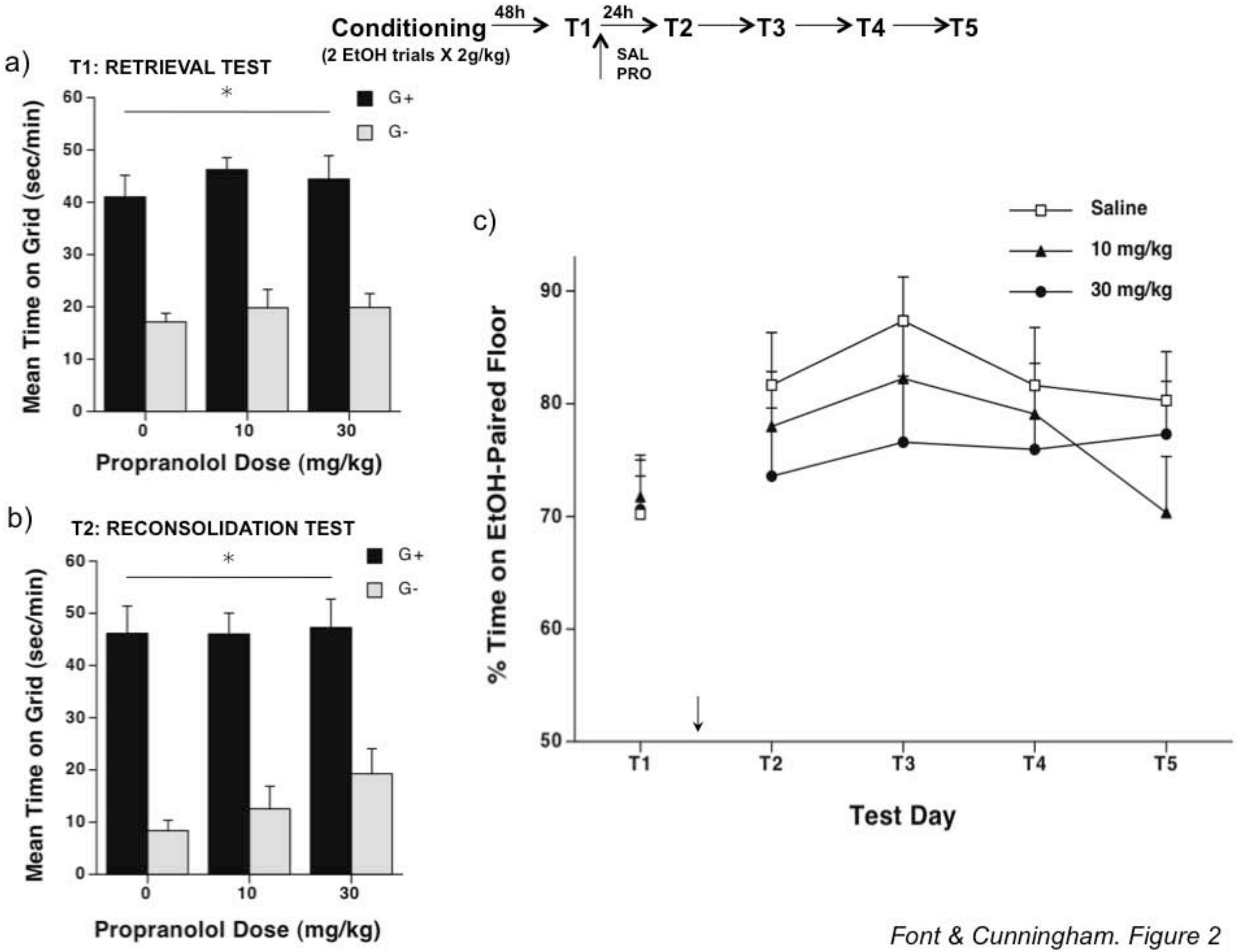
* Denotes a significant main effect of conditioning subgroup but no effect of treatment, $p < 0.01$).

Figure 4. Repeated post-retrieval PRO treatment did not interfere with extinction of ethanol-induced CPP in mice. Performance on 19 post-conditioning tests as percent time spent on the ethanol-paired floor is depicted in Figure 4 as mean ± SEM (n=48). Mice received repeated post-retrieval injections of saline immediately after memory reactivation and PRO 3 h later in their home cages (HC) (Saline-PRO 10 mg/kg) or an immediate injection of PRO and saline 3 hours later (PRO 10 mg/kg-Saline). As it can be seen, both groups expressed long lasting

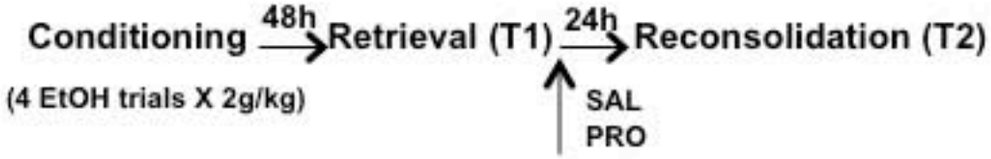
preference for the ethanol-paired compartment that progressively decreased over the course of 19 post-conditioning tests (T1-T19).

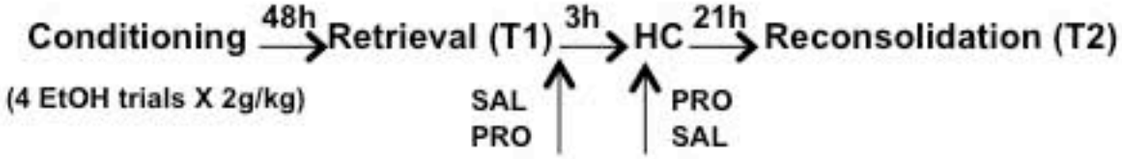


Font & Cunningham. Figure 1

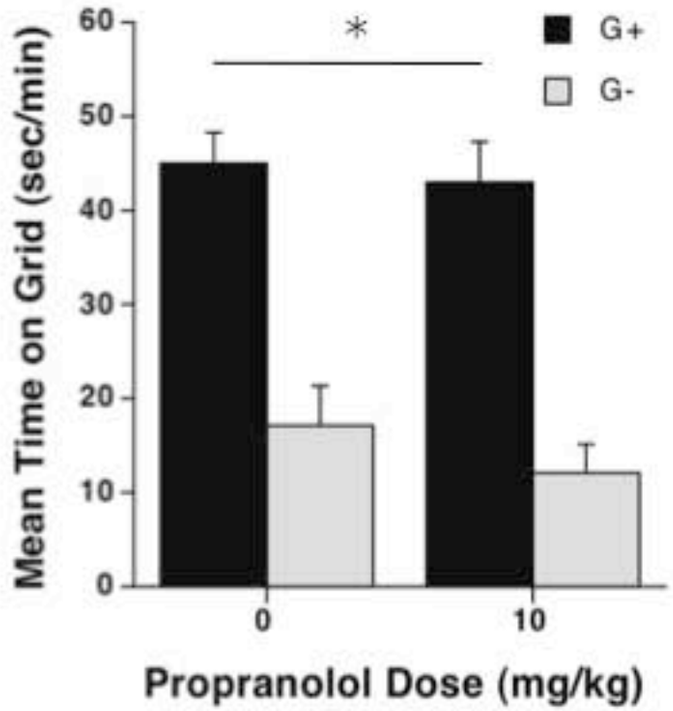


Font & Cunningham. Figure 2

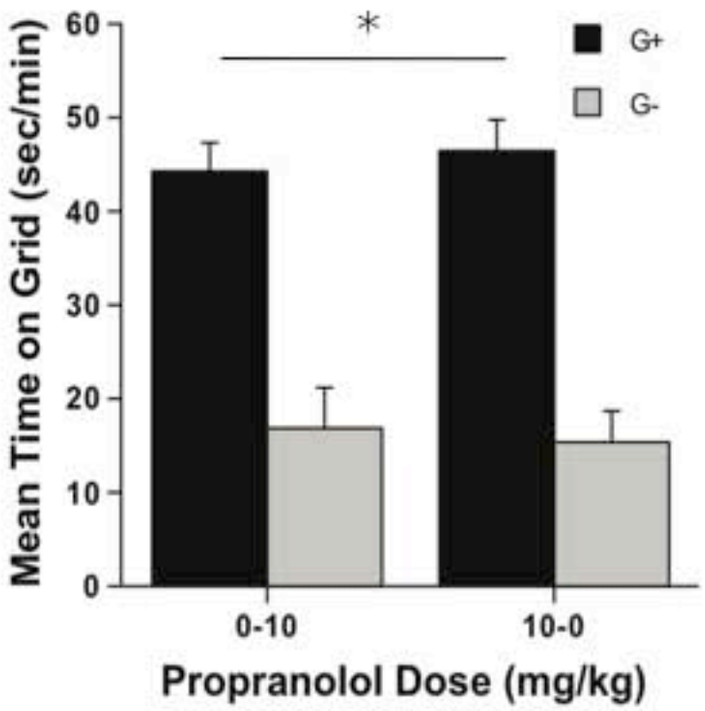




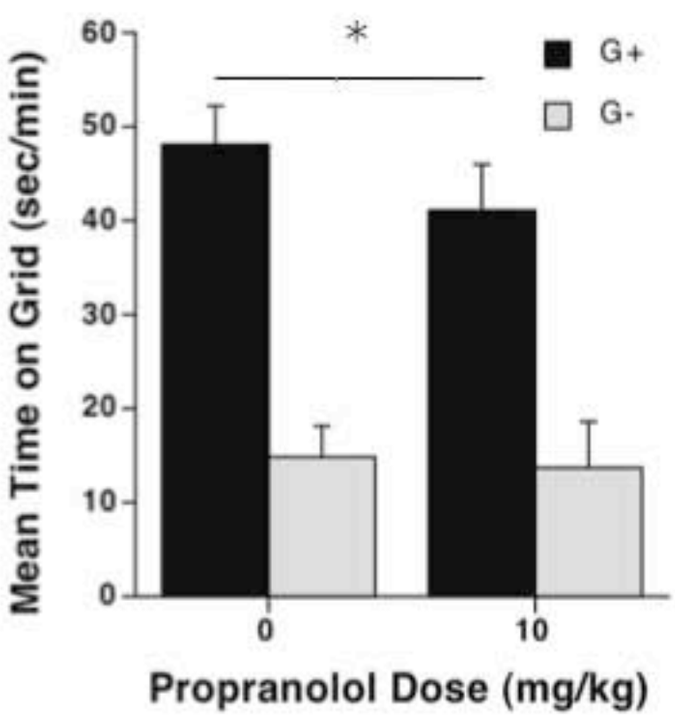
a) T1: RETRIEVAL TEST



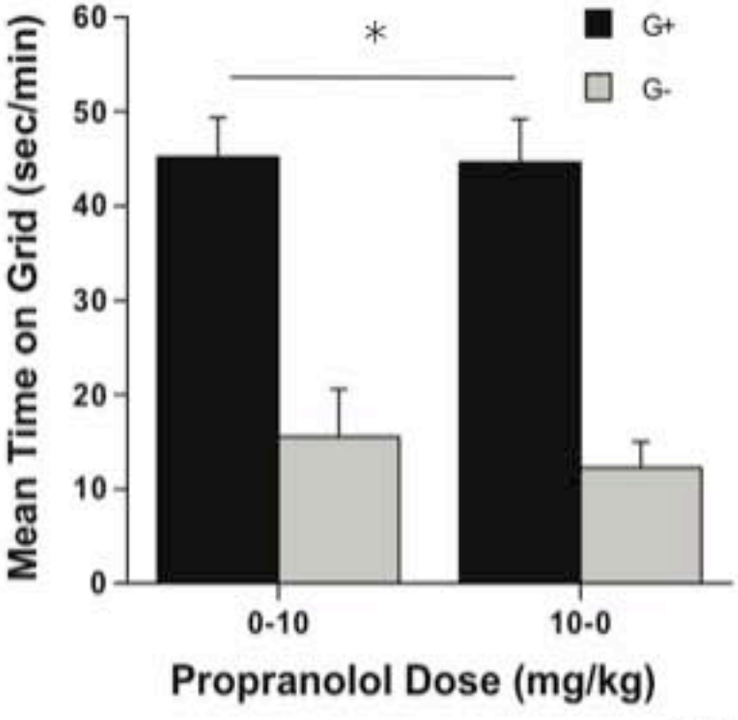
c) T1: RETRIEVAL TEST



b) T2: RECONSOLIDATION TEST



d) T2: RECONSOLIDATION TEST



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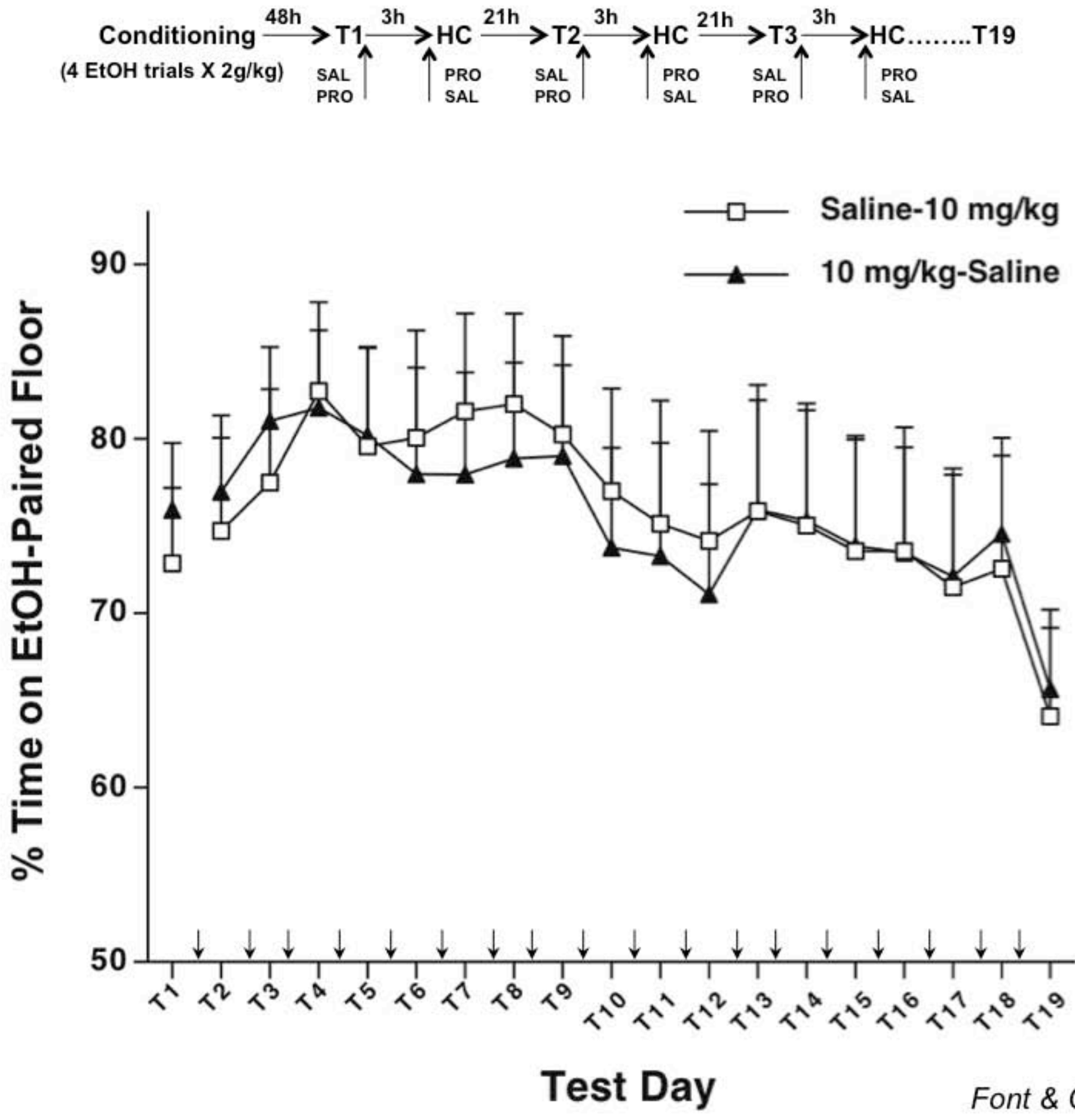

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