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Research report

Reconsolidation of a morphine place preference: Impact of the strength and age of memory on disruption by propranolol and midazolam

M.J.F. Robinson, K.B.J. Franklin*

Department of Psychology, McGill University, 1205 Dr Penfield Ave, Montreal, Quebec, Canada H3A 1B1

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ABSTRACT

Reactivation of memories may render them labile and subject to disruption by amnestic drugs thus reducing their impact on future behavior, but whether it is possible with well-established memories is not known. Here we examined the effect of two amnestic agents on reconsolidation of a conditioned place preference (CPP) for morphine when memory strength and memory age were varied. In a three-compartment apparatus animals received 4 or 8 experiences of morphine in one compartment and saline in the alternative compartment. The memory was then reactivated drug-free, and immediately afterwards animals received an injection of propranolol (10 mg/kg, SC), midazolam (1 mg/kg, IP), both amnestic agents combined, or saline. Animals conditioned with 4 pairings were re-tested 2 and 7 days after reactivation. After conditioning with 8 drug experiences memories were reactivated and treated 8 times, once every 48 h, beginning 1 or 30 days after training. Propranolol, midazolam and their combination, disrupted reconsolidation for weak memories (4 pairings), but had little effect on stronger memories (8 pairings) reactivated 1 day after training. Extending the reactivation-amnestic treatments to 8 sessions did not disrupt the strong memory. Delaying reactivation sessions by 30 days enabled all three amnestic treatments to disrupt reconsolidation. Repeating amnestic treatment appeared to increase the effect of midazolam, but combining propranolol and midazolam did not enhance the amnestic effect. The amount of training and the age of the memory may be boundary conditions for reconsolidation.

1. Introduction

Reconsolidation is the process by which previously consolidated memories are rendered labile and susceptible to disruption following recall. It is increasingly recognized as a phenomenon that applies to a broad range of paradigms and species [1]. There are, however, reports that some memories do not undergo reconsolidation, or that there are conditions which protect them from disruption. Factors which appear to determine the lability of a reactivated memory include, memory age [2–5], training strength [6–8], and the content and duration of the reactivation session [2,9,10]. These limiting factors are regarded as boundary conditions for reconsolidation and are becoming a new focus of reconsolidation research [11]. It remains to be determined whether these boundary conditions are rigid or modifiable. Recently some of the requirements for disrupting robust fear memories have been described [5], but whether the same boundary conditions apply to appetitive memories is not known.

The possible application of reconsolidation as a therapeutic treatment for pathological memories requires a detailed understanding of the boundary conditions as well as finding amnestic agents that can be safely administered to humans. Drugs such as propranolol [12–16] and midazolam [2,17] have been reported to disrupt reconsolidation, and are already approved for clinical use in humans. Post-traumatic stress disorder (PTSD) has been the first therapeutic target [18], but an increasing amount of attention is focused on the possibility of blocking appetitive memories that underlie drug seeking [12,16,19,20]. The most widely used models of reward-related memory in animals are the self-administration paradigm and the conditioned place preference (CPP). Both paradigms have revealed reconsolidation effects for drugs [16,20,21] and natural rewards [19,22,23].

Cues associated with addictive drugs have the ability of inducing strong physiological responses and intense craving for many years following recovery and have been linked with high rates of relapse [24–26]. The CPP paradigm tests the impact that contextual cues may have on drug-seeking, and on cue-elicited craving in a drug-free animal. Beta-adrenergic receptors are known to play a role in memory storage [27,28]. Memory reconsolidation for a drug conditioned place preference can be disrupted by post-reactivation injections of the adrenergic beta-receptor antagonist, propranolol, and the effect is dependent on prior memory reactivation [12,16]. Another class of potential amnestics is the GABA(a) agonists [29]. Midazolam has been shown to disrupt reconsolidation of fear

* Corresponding author. Tel.: +1 514 398 6081; fax: +1 514 398 4896.
E-mail address: keith.franklin@mcgill.ca (K.B.J. Franklin).

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conditioning [2,17], but it has not been tested on the reconsolidation of a drug-induced CPP. Furthermore, previous research that examined reconsolidation in the CPP focused on memories established by 3–5 drug-context pairings [12,16,30–32]. The strength of such memories is minimal in comparison to the number of associations that occur in the normal course of drug addiction. It is not clear whether stronger, well-trained memories still undergo reactivation-dependent reconsolidation that can be blocked by currently available amnestic agents. If stronger memories are less labile to amnestic treatments it is possible multiple reconsolidation treatments and/or a longer conditioning to reactivation interval, would allow the memories to weaken and become labile.

The use of repeated reactivation treatments has previously been examined for both cocaine [30] and amphetamine [33] CPP. Fricks-Gleason and Marshall showed that post-reactivation propranolol caused a loss of preference for the drug-paired context which was more effective following repeated amnestic treatments [30]. Sadler et al. used the NMDA receptor antagonist MK-801 to disrupt the reconsolidation for an amphetamine place preference. They found that multiple reactivation treatments were required for the memory to be disrupted, and for the effect to last up to 10 days after the last reactivation treatment [33].

The effect of memory age on reconsolidation has been controversial. Several studies have suggested that recent memories are more susceptible to memory reconsolidation effects [3,4], or that older memories may require larger doses of amnestic treatment to block reconsolidation of fear conditioning [2]. On the other hand it has been reported that well-trained memories are labile only after a long training to reactivation interval [5].

In this study we explored the effect of amount of training on the susceptibility of a morphine-induced CPP to reactivation-dependent amnestic effects by two agents—propranolol and midazolam. We also examined whether amnestic effects could be potentiated by combining amnestic agents, administering repeated treatments, or by introducing a delay between conditioning and reactivation [5].

2. Materials and methods

2.1. Animals

Subjects were male Long Evans rats (125–150 g) from Charles River, St Constant, Quebec, Canada. Rats were individually housed in a colony room, maintained on a 12 h light–dark cycle (lights on 7 am) with a constant temperature of approximately 21 °C, and had food and water available ad libitum. This research was reviewed by the Animal Ethics Committee of McGill University and carried out in accordance with the guidelines of the Canadian Council on Animal Care.

2.2. Apparatus

The conditioned place preference (CPP) apparatus consisted of three compartments made of wood. Compartments A and B were identical in size (36 cm × 34 cm × 26 cm). They were located side by side and had shaded plexiglass front walls. Compartment C (20 cm × 14 cm × 28 cm) was attached to the rear of compartments A and B and connected them via guillotine doors in the rear wall of compartments A and B. When the doors were lowered, the rat was confined to one of the larger compartments. When the doors were removed, the rat could move freely between compartments A and B via compartment C. The floor of compartment A was painted white and was covered with a large wire mesh flooring (1.2 cm mesh), its ceiling was painted black, and there were black and white vertical stripes on the walls; the floor and ceiling of the other compartment were painted black, with a small wire mesh flooring (0.6 cm mesh), and there were black and white horizontal stripes on the walls. Each large conditioning box contained a Passive Infrared Motion Sensor (Radioshack, 49-208A) with a 180° horizontal detection field, and there were light beam sensors on the entrance of the third compartment. The sensors were connected to a computer which calculated the position of the animal at all times.

2.3. Place conditioning procedure

Animals were weighed and handled daily, beginning at least 3 days before the first training session. Training sessions were separated by 24 h. On the first day of training animals were introduced to the apparatus via box C and allowed to explore freely all three boxes for 30 min. Time spent in each compartment was recorded, and was used to verify that the rats did not exhibit any spontaneous preference.

On each conditioning day the rat was brought to the test room, injected (SC) with the drug (or vehicle) and immediately confined to one of the large compartments for 30 min. On alternate days, the rat was injected with the vehicle (or drug), and confined for 30 min to the other compartment. The order of injection (drug or vehicle) and the compartment paired with the drug (A or B) was counterbalanced within each group. On test days each rat was introduced via the alley box (box C) and allowed to move freely in all three boxes for 30 min. Time spent in each compartment was recorded.

2.4. Experiment 1: the effect of propranolol, midazolam, and their combination on reconsolidation

During training, rats received 4 pairings of morphine with one compartment and 4 pairings of vehicle with the other compartment. The day following the last training session the memory was reactivated by a test for a CPP. Each rat was introduced via the alley box (box C) and allowed to move freely in all three boxes for 30 min. Time spent in each compartment was recorded. Immediately after this reactivation session rats received an injection of propranolol (SC), midazolam (IP), both drugs, or vehicle. An additional group was administered the combination of propranolol plus midazolam without reactivation. The non-reactivated control group was brought to the testing room and weighed, but was not introduced into the apparatus before receiving its injection of propranolol and midazolam. All animals were re-tested 2 and 7 days later to see if the memory for the CPP had reconsolidated. A morphine-primed test session was given 72 h after the 1 week test, to examine whether drug exposure could reactivate the CPP. Rats were given 5 mg/kg morphine (SC) immediately before the test. The design of Experiment 1 is summarized in Fig. 1.

2.5. Experiment 2: reconsolidation of a strong morphine place preference reactivated after 1 day

The second experiment differed from the first in that rats were given 8 (rather than 4) pairings of drug with one compartment and vehicle with the other compartment. Also a non-reactivated group was not included.

The reactivation protocol, as described in Experiment 1 and summarized in Fig. 1, began 1 day after the last conditioning session and was repeated 8 times at 48 h intervals. Each reactivation session doubled as a test of the effect of the previous treatment on reconsolidation of the memory. A morphine-primed (5 mg/kg, SC) test session was given 48 h after the 8th reactivation/test session to examine whether drug exposure could reactivate the CPP.

2.6. Experiment 3: reconsolidation of a strong morphine place preference reactivated after 30 days

The third experiment differed from the second in that rats were returned to their home cage for 30 days after the 8th conditioning session. After 30 days all groups underwent the same reactivation and reconsolidation testing protocol as in Experiment 2. The design is summarized in Fig. 1.

2.7. Experiment 4: the effect of repeated non-reactivated propranolol injections on a morphine place preference

Experiment 4 was a control experiment to confirm that repeated injections of propranolol without reactivation did not disrupt a CPP. Animals received 4 pairings of drug with one large compartment and vehicle with the other. On each of the 4 days after training, animals were brought to the testing room and weighed, but were not introduced into the apparatus before they received an injection of propranolol (10 mg/kg, SC). Animals were then tested drug-free on the fifth day.

2.8. Drugs and injections

Morphine (Sabex, Quebec) was diluted to 5 mg/ml in 0.9% sodium chloride and given (SC) at a dose of 1 ml/kg. Saline was used for control injections in the same volume.

Propranolol (Sigma–Aldrich, USA, Ltd.) was dissolved in 0.9% sodium chloride and given (SC) at a dose of 10 mg/kg. Midazolam (Sandoz, Canada, Inc.) was provided in vials of 5 mg/5 ml and injected (IP) at a dose of 1 mg/kg. Controls received an equivalent volume of saline.

2.9. Statistical analysis

Data collected during pre-exposure and test/reactivation sessions consisted of time spent in seconds in each of the two large chambers in the apparatus. The time spent in the third compartment was not analyzed. Animals which did not display a positive preference (time spent in drug-paired compartment minus time spent in saline-paired > 0) for the drug-paired compartment on initial reactivation were excluded from the analysis (Experiment 1: 2 out of 54; Experiment 2: 5 out of 48; Experiment 3: 9 out of 51).

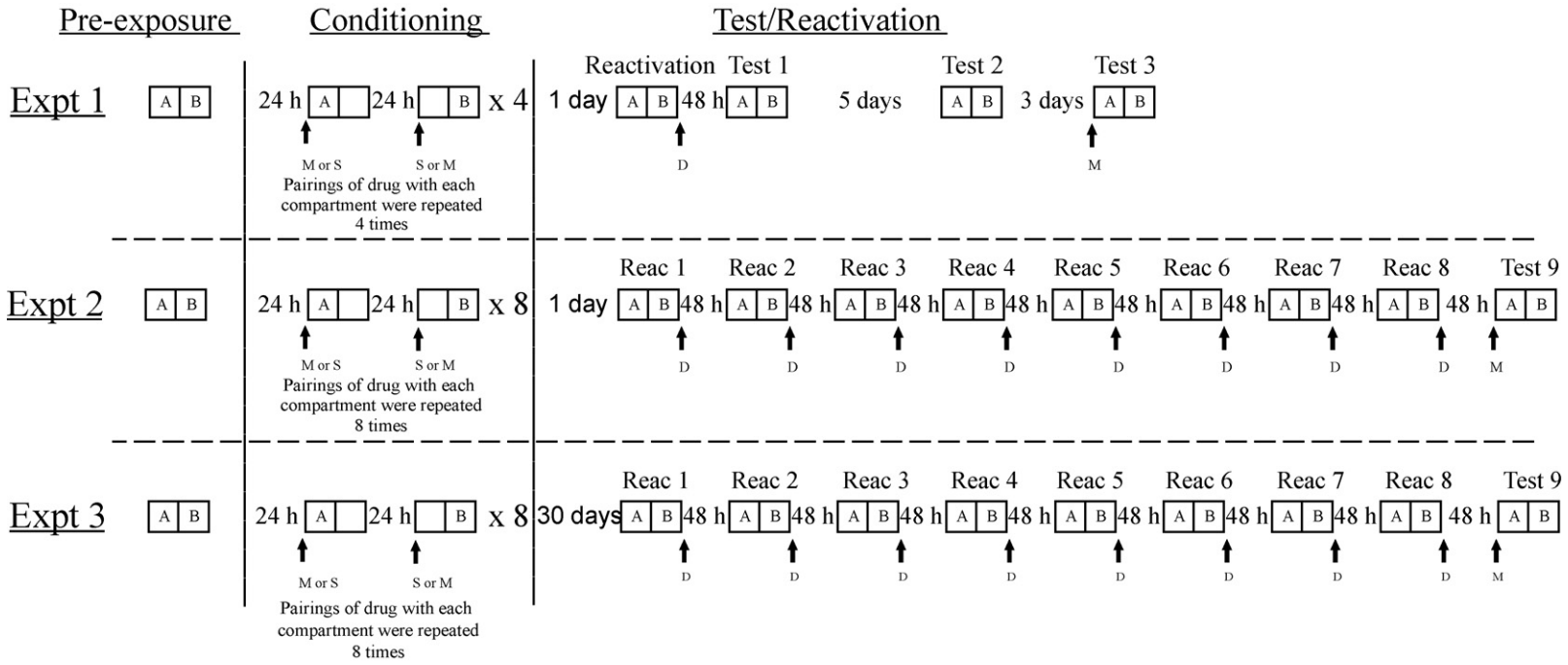

Fig. 1. Summary diagram of the design and the sequence of procedures in Experiments 1–3. M = morphine (5 mg/kg, SC); S = saline; D = post-reactivation drug treatment; A and B denote the compartment(s) the animal has access to during that session, compartment C is not shown. During conditioning the order of injection and compartment paired with the drug were counterbalanced.

Not all groups have scores for the reactivation session, so there is no possible complete factorial design for Experiment 1. We used 2 strategies for analysis of reconsolidation effects. We first examined whether each group showed a significant preference for the drug-paired over the vehicle-paired compartment on each trial. Only a subset of the possible between cell comparisons are meaningful so we used a priori contrasts to maximize power and reduce the risk of Type II errors [16]. Note that this strategy is conservative because incorrectly accepting the null hypothesis for the place preference would increase the probability of reporting a reconsolidation block where none was present. The ANOVA (Statistica) was with one repeated measure (the time each animal spent in either compartment). Since morphine is known to produce a CPP, and it was predicted that all groups would prefer the morphine-paired side, thus significance tests for the CPP were one-tailed ($p = .05$).

Second, we explored whether there were any significant changes in preference within treatment groups from the first reactivation day to each subsequent test day. The ANOVA (Statistica) was with one repeated measure, comparing the preference score (time spent in drug compartment minus time in saline compartment) on the initial reactivation day against the preference score on each subsequent test/reactivation day. Since both increases and decreases in preference were of interest these comparisons were two-tailed ($p = .05$).

Finally, an ANOVA comparing the time spent in the left vs. the right compartment for each group was run on the pre-exposure session for each experiment to confirm the apparatus was unbiased.

3. Results

3.1. Experiment 1: effect of propranolol, midazolam, and their combination on reconsolidation

When rats were first given an opportunity to explore the apparatus before training the 52 subjects showed no preference for either compartment ($F(4,47) = 0.412$, NS), confirming the apparatus was unbiased.

Following conditioning all four reactivated groups displayed a significant conditioned place preference (CPP) (Fig. 2) for the morphine-paired compartment prior to the amnestic treatments (saline: $F(1,36) = 28.652$, $p < .05$; propranolol: $F(1,36) = 23.187$, $p < .05$; midazolam: $F(1,36) = 28.167$, $p < .05$; propranolol + midazolam: $F(1,36) = 41.895$, $p < .05$). There was no significant difference in preference on initial reactivation between these four groups ($F(3,36) = 0.985$, NS).

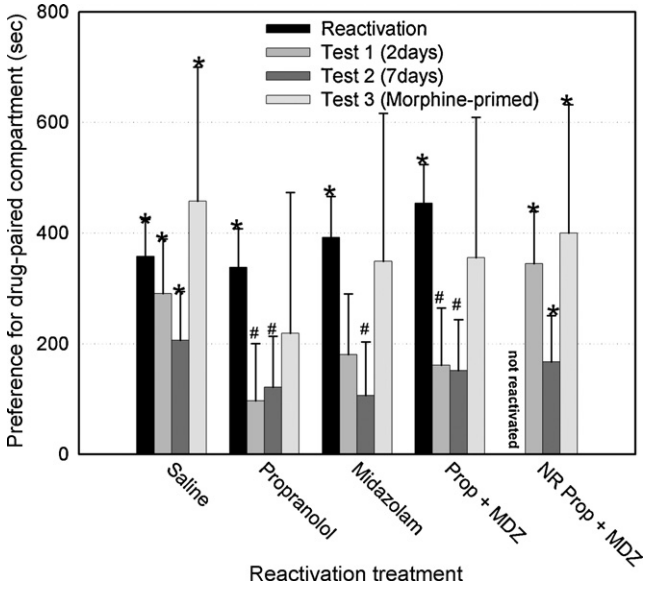

Fig. 2. Effect of propranolol (10 mg/kg; $N = 10$), midazolam (1 mg/kg; $N = 9$), propranolol + midazolam (10 mg/kg, 1 mg/kg; $N = 10$) or saline ($N = 11$) given post-reactivation, or propranolol + midazolam (10 mg/kg, 1 mg/kg; $N = 12$) without reactivation, on the expression of a morphine-induced place preference during the initial reactivation, re-tests after 2 or 7 days and a morphine-primed test at 9 days. Data is the time spent in the morphine-paired compartment minus the time spent in the saline-paired side. Vertical bars are standard errors. *Time spent on the morphine-paired side > time spent on vehicle-paired side ($p < .05$). #Size of CPP different from initial reactivation ($p < .05$).

When all groups were tested for the reconsolidation of the CPP 48 h after the reactivation treatment the groups that received saline post-reactivation, or propranolol + midazolam without reactivation, both showed a significant preference for the morphine-paired compartment (saline: $F(1,47) = 8.659$, $p < .05$; non-reactivated propranolol + midazolam: $F(1,47) = 13.315$, $p < .05$), whereas the groups treated with propranolol, midazolam or propranolol + midazolam, showed no preference for the compartment paired with morphine (propranolol: $F(1,47) = 0.882$, NS; midazolam: $F(1,47) = 2.741$, NS; propranolol + midazolam: $F(1,47) = 2.431$, NS). In addition, both the propranolol and the propranolol + midazolam groups displayed a significant decrease in the size of the CPP between initial reactivation and the first reconsolidation test (propranolol: $F(1,36) = 4.901$, $p < .05$; propranolol + midazolam: $F(1,36) = 7.254$, $p < .05$).

When animals were re-tested after 7 days (Fig. 2), the saline-treated group still displayed a significant CPP (saline: $F(1,47) = 5.551$, $p < .05$), as did the non-reactivated group (non-reactivated propranolol + midazolam: $F(1,47) = 3.989$, $p < .05$). The three active treatment groups did not display a significant CPP (propranolol: $F(1,47) = 1.759$, NS; midazolam: $F(1,47) = 1.209$, NS; propranolol + midazolam: $F(1,47) = 2.729$, NS). In these three groups the size of the CPP was reduced compared to their preference on the initial reactivation test (reactivation vs. reconsolidation test: propranolol: $F(1,36) = 5.703$, $p < .05$; midazolam: $F(1,36) = 9.001$, $p < .05$; propranolol + midazolam: $F(1,36) = 11.176$, $p < .05$).

Three days later, all five groups were given a morphine-primed test (Fig. 2). Only the saline-treated and non-reactivated propranolol + midazolam-treated groups displayed a significant CPP ($Fs(1,47) > 2.972$, $p < .05$). Though the groups treated with amnestics did not show a significant CPP, their preference was not significantly reduced from the initial reactivation ($Fs(1,36) < 0.261$, NS).

3.2. Experiment 2: reconsolidation of a strong morphine place preference reactivated after 1 day

The 43 rats which made up the four groups showed no consistent preference towards either compartment prior to conditioning ($F(3,39) = 0.448$, NS).

After conditioning with 8 drug experiences, all groups displayed a significant CPP (saline: $F(1,39) = 24.010$, $p < .05$; propranolol: $F(1,39) = 37.826$, $p < .05$; midazolam: $F(1,39) = 31.962$, $p < .05$; propranolol + midazolam: $F(1,39) = 46.840$, $p < .05$) as can be seen in Fig. 3, and the size of the CPP did not differ between them ($F(3,39) = 0.63$, NS).

It can be seen in Fig. 3 that 48 h after an amnestic (or control) treatment, all four groups still displayed a significant CPP (saline: $F(1,39) = 10.548$, $p < .05$; propranolol: $F(1,39) = 29.877$, $p < .05$; midazolam: $F(1,39) = 12.894$, $p < .05$; propranolol + midazolam: $F(1,39) = 14.949$, $p < .05$). Over repeated reactivations and reconsolidation tests, saline-treated rats retained a significant CPP (saline: $Fs(1,39) > 3.683$, $p < .05$) until the 8th reactivation ($F(1,39) = 2.186$, NS) but the size of the CPP was not significantly reduced from the initial CPP ($F(1,38) < 1.0$, NS). The propranolol-treated group also retained a stable CPP until the 7th reactivation (reactivation 7: propranolol: $F(1,39) = 1.316$, NS). The CPP of the group given midazolam became unreliable following the 3rd reactivation (reactivation 4: midazolam: $F(1,39) = 2.452$, NS) but did not become significantly different from the initial reactivation 1 by the 8th test ($Fs(1,39) < 3.512$, NS). The combined treatment group also lost the CPP after the 3rd reactivation (propranolol + midazolam: $F(1,39) = 1.518$, NS) but it seemed to recover, and was still

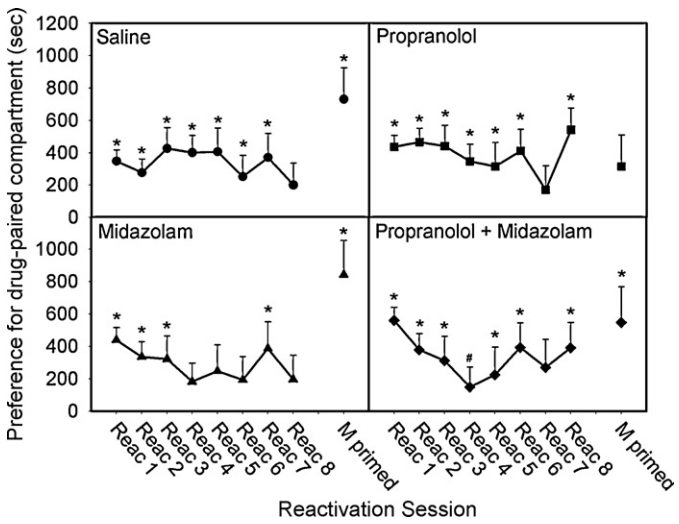

Fig. 3. Effect of propranolol (10 mg/kg; $N = 12$), midazolam (1 mg/kg; $N = 10$), propranolol + midazolam (10 mg/kg, 1 mg/kg; $N = 9$) or saline (N = 12) given post-reactivation on the expression of a strong morphine-induced place preference reactivated 1 day after training. Data is the time spent in the morphine-paired compartment minus time spent in the vehicle-paired compartment on reactivations 1–8, or following a morphine-primed test (Test 9). Vertical bars are standard errors. *Time spent on the morphine-paired side > time spent on vehicle-paired side ($p < .05$). #Size of CPP different from initial reactivation ($p < .05$).

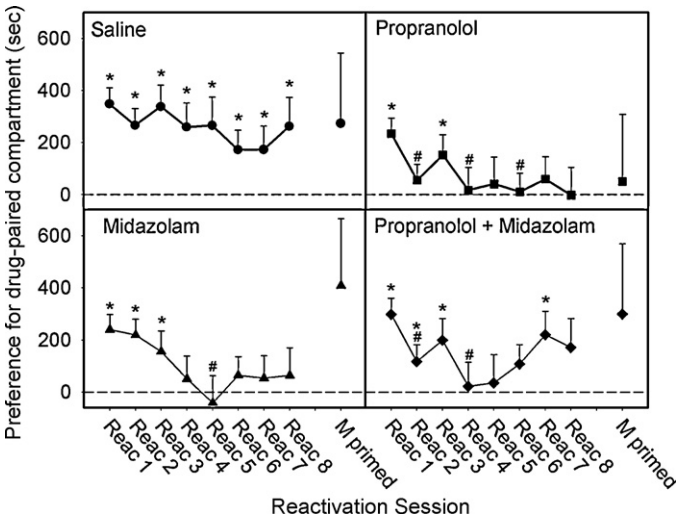

Fig. 4. Effect of propranolol (10 mg/kg; $N = 11$), midazolam (1 mg/kg; $N = 11$), propranolol + midazolam (10 mg/kg, 1 mg/kg; $N = 10$) or saline ($N = 10$) given post-reactivation on the expression of a strong morphine-induced place preference reactivated 30 days after training. Data is the time spent in the morphine-paired compartment minus time spent in vehicle-paired compartment on reactivations 1–8, or following a morphine-primed test (Test 9). Vertical bars are standard errors. *Time spent on the morphine-paired side > time spent on vehicle-paired side ($p < .05$). #Size of CPP different from initial reactivation ($p < .05$).

significant on the 8th reactivation (propranolol + midazolam: $F(1,39) = 6.224$, $p < .05$).

On the morphine-primed test (Fig. 3) the saline, midazolam and propranolol + midazolam groups displayed a significant preference for the drug-paired compartment (saline: $F(1,39) = 14.338$, $p < .05$; midazolam: $F(1,39) = 15.897$, $p < .05$; propranolol + midazolam: $F(1,39) = 6.026$, $p < .05$) but the group that received repeated propranolol injections did not show a CPP (propranolol: $F(1,39) = 2.667$, NS). None of the groups displayed a CPP that was significantly different from their initial preference ($Fs(1,39) < 3.340$, NS).

3.3. Experiment 3: reconsolidation of a strong morphine place preference reactivated after 30 days

The 42 rats showed no spontaneous bias towards either compartment during pre-exposure ($F(3,38) = 0.138$, NS).

Following conditioning with 8 drug and 8 saline experiences, rats were left in their home cages for 30 days before reactivation tests began. Each reactivation session was followed immediately by injections of propranolol, midazolam, propranolol + midazolam or saline. On the first reactivation all groups displayed a significant preference (Fig. 4) for the morphine-paired compartment (saline: $F(1,38) = 31.729$, $p < .05$; propranolol: $F(1,38) = 15.800$, $p < .05$; midazolam: $F(1,38) = 16.506$, $p < .05$; propranolol + midazolam: $F(1,38) = 23.187$, $p < .05$), and there was no significant difference between these four groups ($F(3,38) = 0.223$, NS). On the first reconsolidation test (2nd reactivation) the groups having received saline, midazolam or midazolam plus propranolol displayed a significant CPP (saline: $F(1,38) = 17.280$, $p < .05$; midazolam: $F(1,38) = 12.879$, $p < .05$; propranolol + midazolam: $F(1,38) = 3.388$, $p < .05$) but propranolol-treated animals no longer exhibited a CPP ($F(1,38) = 0.370$, NS). In addition, the preference in the propranolol and propranolol + midazolam groups was significantly smaller on the reconsolidation test than on the previous reactivation test (propranolol: $F(1,38) = 5.310$, $p < .05$; propranolol + midazolam: $F(1,38) = 4.877$, $p < .05$).

Saline-treated subjects retained a strong CPP through 8 reactivations (saline: $Fs(1,38) > 3.702$, $p < .05$) the size of which did not diminish from the first to the 8th reactivation ($F(1,38) < 3.07$, NS). In contrast, propranolol-treated animals lost the CPP after one propranolol-treated reactivation, regained it on the next trial ($F(1,38) = 3.742$, NS), and then lost it again ($Fs < 0.387$, NS). They showed a significant shift in preference from the initial reactivation on reactivation sessions 2, 4 and 6 ($Fs(1,38) > 4.651$, $p < .05$). Likewise, midazolam-treated subjects ceased to show a significant CPP after three amnestic treatments ($Fs(1,38) < 0.339$, NS), and the CPP was significantly smaller on reactivation 5 ($F(1,38) = 5.085$, $p < .05$). The propranolol–midazolam combination treatment also reduced the CPP after three treatments (reactivations 4–6: $F(1,38) < 2.066$, NS) but some recovery occurred on the 7th reactivation ($F(1,38) = 6.004$, $p < .05$). Their CPP was reduced on sessions 2 and 4 compared to the initial reactivation ($Fs(1,38) > 4.877$, $p < .05$).

When the groups were tested morphine primed, 48 h after the 8th reactivation, the time spent in the drug-paired compartment increased for most groups, but none of them showed a significant CPP (Test 9: saline: $F(1,38) = 1.031$, NS; propranolol: $F(1,38) = 0.040$, NS; midazolam: $F(1,38) = 2.536$, NS; propranolol + midazolam: $F(1,38) = 1.235$, NS).

3.4. Experiment 4: the effect of repeated non-reactivated propranolol injections on a morphine place preference

The pre-exposure revealed no spontaneous preference for either compartment ($t(7) = 0.445$, NS).

After 4 pairings of morphine with one compartment and 4 pairings of vehicle with the other compartment, animals were brought to the testing room and injected with propranolol without memory reactivation once a day for 4 days. On the fifth day, a drug-free reactivation test was run, and it showed animals had acquired a significant CPP ($t(7) = 3.023$, $p < .05$).

4. Discussion

The present experiments showed that a morphine CPP induced by 4 experiences of morphine in one compartment and saline in the other was disrupted by reactivation of the CPP followed by administration of the beta-blocker propranolol, or the GABA(a) agonist

midazolam, or both. The disruption of the place preference was sustained after a week. The disruption of the CPP was reactivation-dependent, since an injection of propranolol + midazolam without prior memory reactivation had no effect on subsequent re-tests. This is also the case for propranolol administered without reactivation (Experiment 4) [16]. In addition, the CPP was not reinstated by morphine priming—a treatment known to precipitate relapse to drug self-administration [34]. When the number of conditioning pairings was increased from 4 to 8, the preference for the drug-paired environment became impervious to a single reconsolidation-blocking treatment. Even repeated reactivations, each followed by administration of an amnestic agent, failed to consistently disrupt the place preference. However, when the reactivation sessions were delayed by 30 days, post-reactivation treatment with propranolol or midazolam or the combination eliminated the CPP after three amnestic treatments, though there was some recovery after the 6th combined treatment. Experiment 4 showed this was not a side-effect of repeated propranolol injections, since animals that received daily propranolol injections for 4 days without reactivation still displayed a place preference.

These results show that after extended training, memory for a drug-place association becomes less susceptible to reconsolidation block. However, these stronger memories once again become susceptible to disruption when reactivation occurs after a long delay. Likewise, Nader et al. [35] showed that a weak contextual fear memory created by 1 tone-shock pairing underwent reconsolidation when reactivated 1 or 14 days after training. When the number of tone-shock pairings was increased to 10, reconsolidation was disrupted by anisomycin infused into the basolateral amygdala if memory was reactivated 30 or 60 days after training, but not when it was reactivated after only 2 or 7 days [5]. Thus, the susceptibility of conditioned fear memories to reconsolidation-blocking treatments seems to depend on both the strength and age of the memory. In contrast, some previous research on contextual fear memories has suggested that they become increasingly stable and resistant to systemic [4] or to local injections of anisomycin in either the dorsal hippocampus or the anterior cingulate cortex [3]. However, reconsolidation for remote 36-day-old memories could be blocked by anisomycin, if the duration of the reactivation session was extended from 2.5 to 15 min [3]. Suzuki et al. using fear conditioning in mice, found that stronger memories, reactivated 24 h after training, required longer re-exposures to undergo reconsolidation (10, rather than 3 or 5 min) [8]. Similarly, reconsolidation block of a strong memory for sucrose-seeking, established by 12 days of training with 50 CS-US pairings per day, required memory reactivation sessions to occur 3 weeks after training and to last 20 rather than 10 min [19]. These results suggest that there is complex relationship between the strength and age of a memory and the parameters of reactivation sessions that determines whether memory reconsolidation is susceptible to disruption. Nevertheless our results support the idea that although stronger memories may initially be resistant to reconsolidation-blocking treatments, they become once again labile after prolonged disuse.

It is possible that increasing the duration of reactivation for a strong morphine place preference might also make reconsolidation labile. However, the effect of extending reactivation sessions is still contentious. The trace dominance theory [36], suggests that when a conditioned association is reactivated, presentation of a conditioned stimulus alone initiates two competing processes, that of reconsolidation and extinction. It is thought that the nature of the reactivation trial will determine whether reconsolidation or extinction will be disrupted by an amnestic treatment. Bustos et al. [2] have shown that reactivation of a fear memory for either 3 or 5 min 24 h after training, followed by injections of 1.5 mg/kg of midazolam, leads to disruption of reconsolidation on a subsequent re-test. Yet if the reactivation session lasts for 10 min, then the same injection of midazolam leads to a disruption of the consolidation of extinction. Thus, extinction might act as a boundary condition for reconsolidation, whereby the occurrence of the extinction process precludes reconsolidation from being disrupted by amnestic treatments [37]. However, against this interpretation, experiments with conditioned fear have suggested that extinction and reconsolidation can co-occur [38]. Nevertheless, one explanation for the stability of a strong CPP might be that when a strong memory was reactivated directly after training, the reactivation sessions induced extinction rather than reconsolidation, and extinction was disrupted by either propranolol, midazolam or their combination. There are several considerations that make this hypothesis unlikely. First, since the saline control group retained a preference for the morphine-paired compartment, which remained significant for seven tests and did not significantly diminish over eight tests, our results suggest reactivation sessions did not induce behavioral extinction. These results agree with previous findings that the morphine-induced CPP is very resistant to extinction [39]. Second, to the extent that the amnestic drugs had any effect it was to facilitate the disappearance of the strong CPP, not prevent it. There is also evidence that the loss of the CPP does not have the characteristics of extinction. It has been shown that when extinction does occur, the CPP is readily reinstated by morphine priming [39]. With the 1- or 30-day-old strong CPP (Experiments 2 and 3), morphine priming did seem to enhance the CPP in groups treated with midazolam or propranolol + midazolam, though the effect was not significant. This suggests these treatments containing midazolam may have facilitated extinction. However, when the CPP was blocked by post-reactivation propranolol treatment in all the experiments, priming did not reinstate the CPP. In this regard the loss of the CPP following propranolol does not have the characteristic of extinction. It should also be noted that injections of propranolol disrupt the expression of conditioned fear, without interfering with extinction learning [40], suggesting that extinction is not sensitive to propranolol. In light of these considerations, our findings support the interpretation made by Fricks-Gleason and Marshall [30], that the reduction in preference is a result of reconsolidation blockade rather than facilitated extinction, and are consistent with the idea that memory strength and age are determining factors for reconsolidation.

The current study also examined the effect of repeated administrations of different amnestic agents. The results indicated that when strong memories are reactivated following a delay (Experiment 3), repeated post-reactivation treatments progressively weaken the CPP over several trials. This was particularly noticeable with midazolam treatment. In the case of animals receiving propranolol, a single post-reactivation treatment was sufficient to disrupt reconsolidation, although the preference appeared to rebound. In contrast, repeated propranolol treatment without reactivation did not disrupt the CPP in Experiment 4.

A further objective of our study was to examine the impact of combining two amnestic treatments that disrupt memory through different cellular pathways to assess whether amnestic effects might synergize. The β-noradrenergic receptor is a Gs protein coupled receptor that is linked to adenylate cyclase and facilitates the production of intracellular cAMP [41]. It plays a facilitatory role in long-term potentiation (LTP) [27,42]. Whereas benzodiazepines appear to enhance GABA's effects, allowing for increased chloride entry into the cell, thereby leading to hyperpolarization [43,44]. Both treatments would be expected to interfere with the induction of the molecular cascade that leads to memory reconsolidation [29,45–47]. As expected, propranolol disrupted the morphine CPP as has been previously reported [16]. Midazolam also disrupted reconsolidation of a morphine place preference as it does in the fear conditioning paradigm [2,17]. Midazolam can therefore be added to the list of compounds that may possess therapeutic potential for treating addiction. However, the combination of propranolol

and midazolam was not more effective than either drug alone. Rather, the place preference seemed to recover while treatments continued, suggesting that there may be interference between the consequences of activating GABA(a) and blocking beta-adrenergic receptors in regard to reconsolidation. Whether combinations of other amnesic drugs would be effective remains to be determined.

To summarize, our findings suggest that memory strength may determine whether reactivation-dependent destabilization of memory is possible. Strong memories that are resistant to reconsolidation block may once again enter a labile state upon reactivation if the training to reactivation interval is sufficiently long, and repeating reconsolidation-blocking treatments may promote memory disruption.

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