Research report

The effect of propranolol dose and novelty of the reactivation procedure on the reconsolidation of a morphine place preference

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A B S T R A C T

Previously consolidated memories may become labile when they are reactivated and require reconsolidation. It has been suggested that when novel information is present at the time of memory reactivation reconsolidation is engaged but when no new information is present, reconsolidation may not occur, and extinction may be the dominant process instead. To test this idea we trained rats to associate a context with the rewarding properties of morphine (5 mg/kg, sc) over four conditioning pairings. Following training, animals were reactivated by a 30-min test session, once a day for 3 days. Rats were injected with the amnestic drug propranolol (10 or 40 mg/kg, sc) following reactivation either on the first or on the second day. They received saline on the alternate day. Propranolol disrupted reconsolidation for a conditioned place preference only when given on the first reactivation day, and this effect was more robust following the higher dose of propranolol. In contrast, animals given propranolol on the second reactivation day still displayed a preference for the morphine-paired context on the final test day. These results support the view that for memory to return to a labile state, the situation that evokes reactivation needs to be novel in some way. If the reactivation situation is familiar, reconsolidation may not occur.

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An optimal memory storage process needs to balance the ability to form complicated associations which may remain unchanged over many years with the potential for plasticity when relevant new information is available [1–4]. The neural mechanisms which underlie these properties of memory have come to be referred to as consolidation and reconsolidation, respectively [5,6].

The typical reconsolidation experiment involves a reactivation session that differs from initial conditioning in that the conditioned stimulus (CS) is presented alone, without the unconditioned stimulus (UCS) [6]. This also constitutes an extinction trial, and repeated presentation of CS-no US will lead to loss of the conditioned response (CR) [7]. Extinction does not remove the previously consolidated memory, but instead creates a new CS-no US memory [7,8] which is also susceptible to amnestic agents such as protein synthesis inhibitors [3], benzodiazepines [1] and beta-blockers [9]. The issue that arises is why do amnestic agents sometimes block extinction and sometimes block reconsolidation? It has been suggested that reconsolidation and extinction are competing processes at the time of reactivation, and that the parameters of the memory and the reactivation session will determine which of the two becomes the dominant process [1–3] which will be disrupted by amnestic treatment. Some of the parameters that seem to be involved in distinguishing between reconsolidation and extinction relate to the duration and number of re-exposures to the CS, as well as the content of the re-exposure and the type of memory involved [1]. Morris et al. suggested that memories that required the encoding of new information each day, such as a delayed matching-to-place task, were more susceptible to protein synthesis-dependent reconsolidation, than a reference memory where performance had previously reached asymptote [10]. An alternative suggestion is that the trigger for reconsolidation is a mismatch between what is expected according to previous learning and what actually occurs at the time of reactivation [11]. A requirement for new information to trigger reconsolidation has been found for taste-recognition and spatial memory, tasks which do not require daily updating [12,13]. This interpretation implies that when a reactivation situation is repeated and has previously been encountered, little new information is present, and extinction will be more likely than reconsolidation.

We have previously shown that a 30 min test/reactivation session following training of a morphine CPP will induce reconsolidation which can be disrupted by an immediate post-reactivation injection of propranolol (10 mg/kg, sc) [14]. Here we predicted that a second identical reactivation session would be less novel and reduce the extent to which reconsolidation is engaged. If reconsolidation is only weakly engaged then disruption of the memory by post-reactivation propranolol might be prevented or require a larger dose of propranolol.

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 21C, and had food and water available ad libitum. They were handled on 3 days prior to the beginning of experimental procedures.

2. Apparatus

The conditioned place preference (CPP) apparatus [14] consisted of three compartments. 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. 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. 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.

3. Place conditioning procedure

During the place conditioning procedure, all animals were weighed and handled daily. Training days were separated by a 24-h interval. On the first day of training animals were introduced via box C and allowed to explore freely all three boxes for 30 min (pre-exposure). Time spent in each compartment was recorded, and was used to verify that the rats did not exhibit any spontaneous preference for one compartment.

On days 2–9 each rat was brought to the test room, injected (SC) with morphine 5 mg/kg (or vehicle) and immediately confined to compartment A or B for 30 min. On alternate days, the rat was injected with the vehicle (or morphine), 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 days 10–12 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 the first reactivation two groups of rats received propranolol (10 or 40 mg/kg) and three groups received saline. After the second reactivation day the groups that received propranolol on day 10 received saline, while two of the groups that had received saline now received propranolol. The remaining rats received a second injection of saline.

4. Drugs and injections

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

Propranolol hydrochloride (Sigma–Aldrich, USA, Ltd.) was dissolved in 0.9% sodium chloride and given (SC) at two different concentrations: (1) diluted to 10 mg/ml and given at a dose of 10 mg/kg and (2) diluted to 20 mg/ml for a dose of 40 mg/kg because it proved to be insoluble at 40 mg/ml. The dose of 40 mg/kg propranolol is the highest, non-toxic single dose reported

[15] though it has been used orally up to 100 mg/kg/day [16]. (−)Propranolol is an antagonist at the 5HT autoreceptor [17] but only beta-receptors have been implicated in its memory blocking effect [18]. Controls received an equivalent volume of saline.

5. Statistical analysis

Data collected during pre-exposure and test/reactivation sessions consisted of time spent in seconds in each of the three chambers in the apparatus.

We used two strategies for analysis of reconsolidation effects by ANOVA (Statistica). We first examined whether each group showed a significant preference for the drug-paired over the vehicle-paired compartment on each trial. Morphine is known to produce a CPP and it was expected that all groups would prefer the morphine-paired side. Incorrectly accepting the null hypothesis would increase the probability of reporting a reconsolidation block where none was present so we used a priori contrasts to maximize power and reduce the risk of Type II errors and Alpha was set at $p = 0.05$, 1 tail. Animals which did not display a preference for the drug-paired compartment (drug time − saline time > 0) on the first reactivation were excluded from the results. Five out of 54 animals were so excluded.

Second we explored whether there were any significant shifts in preference within treatment groups from the preference on the first reactivation day to each subsequent test day. This hypothesis was not directional so Alpha was set at $p = 0.05$, 2 tail.

To assess the effect of when the reconsolidation treatment was administered, the preference of the four propranolol-treated groups displayed on their pre-propranolol reactivation day was compared to the preference expressed on the following test day.

6. Results

On test 1 following four cycles of conditioning, all five groups displayed a significant place preference for the drug-paired compartment (Group 10 mg/Sal: $F_{(1, 44)} = 28.158$, $p < 0.05$; 40 mg/Sal: $F_{(1, 44)} = 72.278$, $p < 0.05$; Sal/10 mg: $F_{(1, 44)} = 59.902$, $p < 0.05$; Sal/40 mg: $F_{(1, 44)} = 43.668$, $p < 0.05$; Sal/Sal: $F_{(1, 44)} = 47.802$, $p < 0.05$). There was no significant difference between these five groups in the size of the preference on the first reactivation ($F_{(4, 44)} = 1.466$, NS) (see Fig. 1).

Twenty-four hours later, the CPP memory was again reactivated (test 2) to assess the effect of the previous day's treatment on the morphine place preference. The three groups administered with saline following the initial reactivation of the memory still displayed a significant place preference (Sal/10 mg: $F_{(1, 44)} = 10.028$, $p < 0.05$; Sal/40 mg: $F_{(1, 44)} = 5.250$, $p < 0.05$; Sal/Sal: $F_{(1, 44)} = 3.817$, $p < 0.05$). Of the groups injected with propranolol post-reactivation, the CPP was abolished in the group receiving 10 mg/kg but those receiving 40 mg/kg still displayed a preference for the drug-paired compartment (10 mg/Sal: $F_{(1, 44)} = 0.033$, NS; 40 mg/Sal: $F_{(1, 44)} = 3.540$, $p < 0.05$). However, the size of the CPP was significantly reduced from test 1 in both groups (10 mg/Sal: $F_{(1, 44)} = 6.192$, $p < 0.05$; 40 mg/Sal: $F_{(1, 44)} = 5.622$, $p < 0.05$; all other $Fs < 2.25$).

On the final recall test, the three groups injected with saline after test 1 (Sal/10 mg, Sal/40 mg and Sal/Sal), including those injected with propranolol following test 2, again displayed a significant preference for the morphine-paired compartment (Sal/10 mg: $F_{(1, 44)} = 23.557$, $p < 0.05$; Sal/40 mg: $F_{(1, 44)} = 18.558$, $p < 0.05$; Sal/Sal: $F_{(1, 44)} = 16.853$, $p < 0.05$). For the group that received a low dose of propranolol on test 1 and saline on test 2 its original place pref-
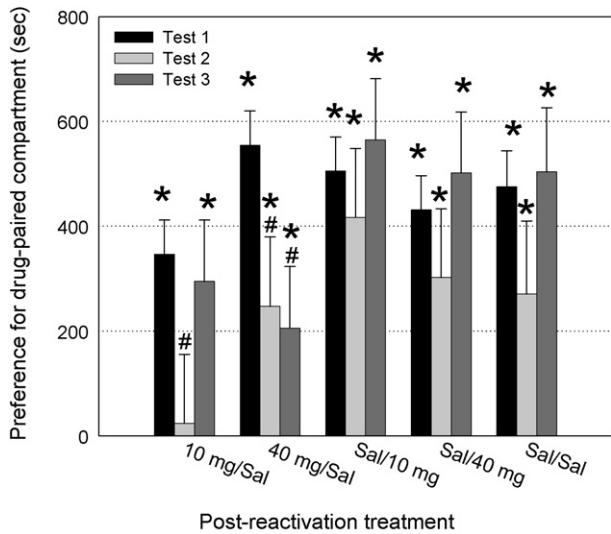
Fig. 1. Size of a morphine-induced place preference before and after propranolol or saline was administered post-reactivation on the first or second place preference test (10 mg/Sal, $N = 10$; 40 mg/Sal, $N = 10$, Sal/10 mg, $N = 10$; Sal/40 mg, $N = 10$, Sal/Sal, $N = 9$). Data is the time spent in the morphine minus vehicle-paired compartments on each of three reactivation/tests. *$p < 0.05$ for morphine vs vehicle-paired; #$p < 0.05$ for reactivation vs test.

erence rebounded (10 mg/Sal: $F_{(1, 44)} = 6.247$, $p < 0.05$) while the group given a high dose of propranolol (Sal/40 mg) still displayed a significant place preference for the drug-paired side 40 mg/Sal: $F_{(1, 44)} = 3.137$, $p < 0.05$). Nevertheless the size of the CPP was still significantly decreased from test 1 (40 mg/Sal: $F_{(1, 44)} = 6.768$, $p < 0.05$).

Fig. 2 shows the effect of propranolol according to whether it was given on the first reactivation or the second reactivation. As can be seen, there was a significant effect of 'day of reactivation' ($F_{(1, 36)} = 9.750$, $p < 0.05$), but no effect of 'propranolol dose' ($F_{(1, 36)} = 0.046$, NS) or day by dose interaction ($F_{(1, 36)} = 0.014$, NS).
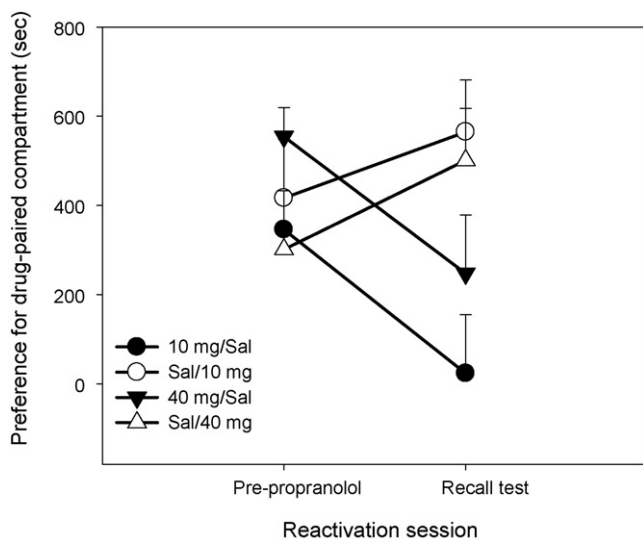

Fig. 2. Comparison of the effect on morphine-induced CPP of propranolol given after the initial reactivation of the CPP (10 mg/Sal, 40 mg/Sal) or after the second reactivation test (Sal/10 mg, Sal/40 mg), on the expression of place preference. Data is the time spent in the morphine minus vehicle-paired compartments on the treatment day vs the following test day.

A comparison of the shift in preference between test 2 and test 3 for the animals treated with propranolol on test 2 (Sal/10 mg and Sal/40 mg) and those given saline on both tests (Sal/Sal) showed no effect of treatment ($F_{(2, 26)} = 0.433$, NS), or treatment by test effect ($F_{(2, 26)} = 0.085$, NS).

During pre-exposure to the apparatus none of the groups displayed a significant preference for one compartment ($F_{(4, 41)} = 1.740$, NS), confirming the apparatus was unbiased, although due to a hardware malfunction no data was collected for 3 out of the 49 rats.

7. Discussion

We found that propranolol administered following reactivation, disrupted memory reconsolidation of a morphine place preference only if the reactivation situation had not been previously encountered. When the amnestic treatment was given following a second reactivation session, there was no effect on reconsolidation. Even a high dose of propranolol did not reduce the preference for the drug-paired compartment when administered after a second test. This argues against the possibility that the reduction in preference seen after the first reactivation is the result of non-specific side-effects of this dose. Similarly it has been reported that if animals were given an untreated test prior to the first reactivation + amnestic treatment, it required up to 10 treatments to disrupt reconsolidation for an amphetamine place preference [19]. These results suggest that a second reactivation session engages the reconsolidation process less efficiently than the initial reactivation. One interpretation is that the mismatch between the information provided by the first reactivation session and the animal's memory of previous encounters with this environment triggers the reconsolidation process to incorporate the new information into the previously consolidated memory [11]. On the first reactivation trial the subject experiences a relatively novel access to both compartments, as well as the absence of morphine in the morphine-paired compartment. Both these experiences are presumably incorporated into the CPP memory by reconsolidation, unless reconsolidation is inhibited by propranolol. On the second trial the situation is unchanged from the first trial but the previously novel elements are now part of the CPP memory. The second reactivation thus provides little mismatch between the reconsolidated memory and the current situation. Similarly, Morris et al. showed that, when memory for a spatial water maze had been trained to asymptote, anisomycin injected into the hippocampus immediately upon reactivation, resulted in no memory disruption in a subsequent test [10]. However, rats that were conditioned with the platform in a different position on each training trial did exhibit a disruption of reconsolidation following a post-reactivation injection of anisomycin. In another study, injections of anisomycin into the CA1 region of the hippocampus only affected reconsolidation of an object recognition memory when the reactivation session comprised a familiar and a novel object [20]. The idea that a mismatch is required for memory to be rendered labile has also recently been supported by findings in humans [21]. Our results thus support the view that the requirement for new information at the time of reactivation is a boundary condition for reconsolidation.

A complementary hypothesis considers the influence of extinction on memory. During a reactivation trial when the CS is presented without the US, the repeated exposure of the CS alone [3,22], or a CS exposure of a longer time duration [3,23], leads to the consolidation of an extinction memory, which masks the presence of the original conditioned memory [7,8]. The extinction memory trace should be active at the same time as the original CR, but how each memory will be affected by administration of an

amnestic treatment is not understood [13,24]. Bustos et al. suggested that the state of the conditioned response at the end of the reactivation session predicts which process will be disrupted by amnestic treatment [1]. Fear-conditioned subjects were given an injection of midazolam after a 5-min reactivation session, which produced a robust conditioned response and high levels of freezing. They found reconsolidation was disrupted on a subsequent re-test. However, when the reactivation duration was increased to 10 min, by which time the conditioned response had begun to extinguish, the subsequent administration of midazolam resulted in a block of extinction consolidation. An experiment by Duvarci et al. [25] supports this interpretation. They trained two different auditory fear memories in the same animal and reactivated both memories, one for 90 min and the other for 30 s, prior to an infusion of anisomycin into the basolateral amygdala. When tested the following day, the animals showed a disruption of reconsolidation for the briefly reactivated memory, but normal extinction for the extensively reactivated memory.

The hypothesis that there is competition between extinction memory and the CR memory does not explain our results. We found no evidence of extinction of the CPP in the groups that did not receive propranolol. The CPP extinguishes slowly [26] and we have previously found that the morphine CPP will sustain eight or more reactivations without extinction [27]. In the present study the fact that there was a strong CPP expressed by the groups given saline on test 1 shows that the CPP memory is dominant on test 2 suggesting that the CR trace will be dominant and reconsolidation should occur.

A further possible explanation might be that saline treatment following the first reactivation allowed those animals to undergo an un-impeded reconsolidation process, thereby producing a more robust and less susceptible memory for reactivation on the second test session [24]. However, it seems unlikely that what is in effect an extinction trial would strengthen the CR.

The possibility that the disruption of reconsolidation on the first test, 24 h after training, is due to propranolol disrupting a late-phase consolidation process can also be rejected. We have shown previously that the memory remains intact when propranolol is given without reactivation [14]. We have also shown that the memory for a morphine-induced CPP can be disrupted by post-reactivation injections of propranolol when the memory is reactivated as long as 30 days after conditioning [27].

The fact that the CPP recovered on test 3 for animals treated with a low but not a high dose of propranolol supports the idea that reconsolidation is not an all or nothing process [28]. The literature indicates that the permanence of reconsolidation block is quite variable [24]. Also amnestic treatment may be less effective at short treatment-testing intervals [29]. We previously reported that 10 mg/kg propranolol following reactivation blocks the reconsolidation for at least 10 days when first assessed 48 h after treatment [14]. However in this experiment, the effect of propranolol treatment was assessed 24 h after the reconsolidation. In fact 10 mg/kg propranolol may have been less effective than it appears, since the scores in the Sal/Sal group suggest some extraneous factor attenuated the CPP on test 2. The idea that disruption of reconsolidation is both dose and time dependent is consistent with the fact that there was no recovery of the CPP after the higher dose of propranolol.

To summarize, we found that the lability of a memory to reconsolidation effects is greatest on the first exposure to an unreinforced reactivation. Familiarity with the situation, or the growth of an extinction memory, seems to render the memory resistant to reconsolidation block by propranolol. Increasing the dose of propranolol does not help overcome this effect but it may reduce the chances of spontaneous recovery.

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