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Research report

Effects of anisomycin on consolidation and reconsolidation of a morphine-conditioned place preference

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Abstract

Protein synthesis inhibitors block consolidation of memory and may also block the reconsolidation of a reactivated memory in paradigms of aversive learning, but the evidence for reconsolidation effects is conflicting in appetitive paradigms. We now report that intra-cerebroventricular (ICV) anisomycin (400 μg) prevents consolidation of morphine-induced place preference (CPP), but does not impair its reconsolidation unless the reactivation procedure associates anisomycin with the morphine context. Rats were injected alternately with morphine (5 mg/kg, IP) or vehicle, and confined to one of two distinctive compartments in a three compartment apparatus. On a subsequent day rats were allowed to choose the compartment they preferred in a 20 min test session. In the first experiment, rats that were injected with vehicle or with anisomycin before or 3 h after training sessions, developed a CPP. However, rats that received anisomycin ICV immediately after training sessions did not develop a CPP. In experiment 2, rats received no ICV injections during initial training. Once a CPP was established, they received four additional training sessions on which they received vehicle or anisomycin ICV. All groups continued to prefer the morphine-paired compartment after reactivation sessions with vehicle or anisomycin ICV. In experiment 3, ICV anisomycin was administered selectively on morphine-paired reactivation trials or saline-paired reactivation trials and the CPP was weakened or strengthened, respectively. This suggests that associations between aversive effects of the amnestic treatment and the morphine context might mimic disruption of reconsolidation.

Keywords: Place conditioning; Morphine; Reward; Memory; Reconsolidation; Consolidation

1. Introduction

Conditioned associations between the effects of an addictive drug and environmental stimuli are believed to play an important role in the maintenance of drug self-administration and the relapse of detoxified drug addicts [1–3]. These associations are very resistant to extinction and to fading with time [4] and they promote relapse after periods of abstinence [5]. If such associations could be weakened it is likely that the success rate of therapy for addictions could be greatly improved. Recent developments in our understanding of memory suggest it may indeed be possible to selectively weaken the impact of some memories on behavior [6].

Memories are initially unstable and are consolidated into a stable and relatively permanent form by a process that depends on the synthesis of new proteins [7–10]. Once consolidated, memories are sustained over long periods of inactivity, but they can become labile when they are reactivated by recall and must then be reconsolidated [11–13]. This reconsolidation process can be disrupted by treatments similar to those that block initial consolidation [6,14].

Evidence for consolidation and reconsolidation derives primarily from experiments on aversive conditioning [6], but there is evidence that initial consolidation of appetitive learning can be blocked by treatments similar to those that block aversive conditioning. Cervo et al. [15] demonstrated that post-training intra-cerebroventricular (ICV) infusions of inhibitors of protein kinase A and protein kinase C block the consolidation of a cocaine conditioned place preference (CPP). Likewise a morphine-induced CPP can be blocked by a metabotropic glutamate receptor antagonist [16] or the CAMKII inhibitor KN-62 [17].

There are now several reports that reconsolidation of a drug-induced CPP can be blocked by amnestic agents but

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the phenomenon has not been consistently replicated. A CPP induced by cocaine is reported to be blocked by post-recall administration of a systemic protein synthesis inhibitor [18], or by an inhibitor of extracellular signal related kinase/mitogen activated protein kinase (MEK) administered either systemically pre-recall [18], or microinjected into the nucleus accumbens core before or after recall [19]. However, Yim et al. found that anisomycin microinjected into the amygdala immediately after recall of a morphine-induced CPP had no effect on the CPP [20]. Three different methods of memory reactivation were tried – exposure to the two sides of the apparatus drug-free, or with morphine or exposure to the morphine-paired side only with morphine – and all three methods produced negative results. In contrast, Milekic et al. reported that anisomycin or cycloheximide injected systemically, or anisomycin microinjected into the basolateral amygdala, hippocampus or nucleus accumbens blocked reconsolidation of the morphine CPP, but only if morphine was administered in the conditioning context at the time of reactivation of the CPP [21]. This result is hard to interpret since an unusual training procedure was used in which animals were trained solely with morphine pairings, and did not experience counterbalanced saline injections in the alternate compartment. However, MEK inactivation with SL327 was also found to block the CPP only if it was administered before mice were re-exposed to morphine in the drug-paired compartment [18]. When reactivation was induced in a drug-free test trial, one study found reconsolidation was blocked [19], another found that expression was blocked but not reconsolidation [18], while two other studies found no amnestic effect [20,21]. When the conditioning drug was present during reactivation but the subject was exposed to both the drug- and vehicle-paired side of the CPP apparatus, memory was not disrupted [20]. Thus, an association between the amnestic agent and the reinforcer seems to be important for reconsolidation effects in drug conditioning. In contrast, the presence of the reinforcer during reactivation is not necessary to block reconsolidation of aversive learning [22]. One possible interpretation of the fact the drug UCS is critical for reconsolidation effects in the CPP, is that an effect of amnestic drugs interacts with some effect of drugs of abuse to disrupt a CPP. Protein synthesis and MAPK are involved in many physiological processes so that inhibiting them is likely to have consequences for brain function in addition to specifically disrupting memory storage. Effects of disordered brain function are likely to be perceived as dysphoriant, and could condition to the reinforcing drug as a discriminative stimulus, or to the context, and thus reduce the tendency of the context to evoke approach behavior [23]. Such devaluation of a reinforcer by anisomycin has been demonstrated in an operant learning paradigm [24].

The aim of the present study was to re-examine whether a morphine-induced CPP is sensitive to the amnestic effects of anisomycin. To avoid overtraining, animals were repeatedly tested to allow the growth of the preference to be tracked. To ensure a reliable CPP, a mid-range dose of morphine was used (5 mg/kg) [25]. The dose of anisomycin was selected at the high end of the scale in order to ensure that any possible amnestic effects would be detected [26]. The first part of this study aimed to confirm that ICV injections of anisomycin reached the sites of appetitive memory by testing their effect on consolidation of a morphine CPP. We then examined the effect of anisomycin on reconsolidation of a morphine CPP using two different methods. The first method examined whether an amnestic effect of anisomycin was apparent when selective associations between the amnestic agent and morphine or the morphine context were prevented by giving anisomycin in conjunction with both conditioning contexts separately. The other aimed to replicate previous findings that anisomycin could block reconsolidation of a morphine CPP when given following a morphine injection in its conditioning context.

2. Materials and methods

2.1. Animals and surgery

Subjects were male Long Evans rats (280–320 g) from Charles River, St. Constant, Quebec, Canada, and were implanted with bilateral (stainless steel 22 GA) cannulas (Plastics One, HRS Scientific, Roanoke, VA) aimed at the lateral ventricles. The anesthetic regimen was pentobarbital (35–50 mg/kg, intraperitoneally, IP), supplemented with ketamine (0.1 ml of l00 mg/ml, intramuscularly, IM) and xylazine (0.1 ml of 5 mg/ml, IM). Stereotaxic coordinates from bregma were A/P: −1.0 (Experiment 1), −0.7 (Experiments 2 and 3); M/L: ±1.5; D/V: −3.8 [27]. Tribrissen (0.1 ml of 24%, sub-cutaneously, SC) was injected pre-operatively to reduce the chance of infection. Atropine sulfate (0.1 ml of 0.5 mg/ml, SC) was given as premedication. Animals were administered dipyrone (l00 mg/kg SC) as an analgesic approximately 2 h following surgery, and were given a minimum of 7 days recovery before testing began. Rats were individually housed in a colony room, maintained on a 12-h light:12-h dark cycle (lights on 7 a.m.) with a constant temperature of approximately 21 °C, and had food and water available ad libitum.

2.2. Apparatus

The 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 tinted 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 1.2 cm wire mesh flooring, its ceiling was painted black and there were black and white vertical stripes on the walls. The floor and ceiling of compartment B were painted black, with a 0.6 cm mesh flooring 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 degree 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

During the place conditioning procedure, animals were weighed and handled daily. Conditioning days were separated by at least 48 h to allow for drug clearance between conditioning trials. Subjects were randomly assigned to groups prior to the first session. On the first day of conditioning animals were introduced via box C and allowed to explore the entire apparatus for 20 min. Time spent in each compartment was recorded.

On each conditioning day the rat was brought to the test room, injected (IP) with morphine (or vehicle) and immediately confined to one conditioning compartment for 20 min. On alternate days, the rat was injected with the vehicle (or morphine), and confined for 20 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

Table 1
Experimental design: arrows indicate that an ICV injection was given in association with the behavioral procedure it is directed at

	Conditioning	Consolidation	Reactivation	Reconsolidation
Experiment 1	M S Test M S ↑ ↑ ↑ ↑	Test	None	None
Experiment 2	MSMS	Test	M S Test M S ↑ ↑ ↑ ↑	Test (48 h and 7days)
Experiment 3	MSMSMS	Test	M **or** S ↑ ↑	Test

In Experiment 1 (Consolidation), ICV infusions were given before (ANI or Vehicle), after (ANI) or 3 h after (ANI). In Experiment 2 (Reconsolidation), ICV infusions were administered before (ANI), after (ANI or Vehicle) or 3 h after (ANI). ICV infusions in Experiment 3 (Morphine- or Saline-paired Reactivation) were given after the reactivation session. *S*, Saline; *M*, Morphine (5 mg/kg). *Note*: The order of morphine and saline pairings was counterbalanced for all experiments but only the first order is shown.

via box C and allowed to move freely in all three boxes for 20 min. The time spent in each compartment was recorded. No drug injections or ICV injections were given on test days.

2.4. Experiment 1: consolidation

In the consolidation experiment, the conditioning consisted of cycles in which the rat was exposed to morphine in one compartment and to vehicle in the other compartment, followed by a test session (see Table 1). On each conditioning day (morphine or saline), rats were given intra-cerebroventricular injections of anisomycin bilaterally. Separate groups of rats received the ICV injections of anisomycin immediately before, immediately after or 3 h after the conditioning session. A fourth group received ICV injections of vehicle immediately before conditioning as a control for the possibility that ICV injections might have aversive effects that could condition to the compartment cues and reduce the size of any preference for the morphine-paired compartment. To reduce the possibility of infection from repeated intracranial injections, rats that lost weight were treated with an antibiotic (Tribrissen: 0.1 ml of 24%, sub-cutaneously). Tribrissen injections were always given more than 12 h before, or 3 h after a training session.

2.5. Experiment 2: reconsolidation

In the reconsolidation experiment, rats first received two cycles of conditioning pairings (drug and vehicle) without any amnesic treatment or intervening tests, followed by a test session to verify that a CPP was established (Table 1). The reconsolidation phase then followed the same procedure used for consolidation in Experiment 1. Rats were given two cycles of conditioning trials to serve as reactivation sessions, where each cycle was followed by a test session. Animals therefore received an ICV injection in association with both compartments (drug- and vehicle-paired). Separate groups of rats received ICV injections of anisomycin immediately before, immediately after or 3 h after the conditioning session. A fourth group received ICV vehicle injections immediately after conditioning as a control for the possibility that the injection procedure might have memory disrupting effects. Forty-eight hours and 7 days after reactivation, all animals were tested to see if the memory for the CPP persisted. As in Experiment 1, rats that lost weight were treated with Tribrissen.

2.6. Experiment 3: morphine- or saline-paired reactivation

In a second reconsolidation experiment, rats first received three conditioning pairings (drug and vehicle) without any amnesic treatment, followed by a test session to verify that a CPP was established (Table 1). The reactivation phase consisted of a single conditioning trial (drug or vehicle) in which one group of rats received morphine on the drug-paired side, while the other group received saline on the vehicle-paired side. Both groups of rats received ICV injections of anisomycin immediately following the reactivation session thereby associating the ICV injections with only one compartment. All animals were tested 48 h later to see if the memory for the CPP persisted. As in Experiments 1 and 2, rats that lost weight were treated with Tribrissen.

2.7. Drugs and injections

In all experiments, morphine (Sabex, Quebec), diluted in 0.9% NaCl to 5 mg/ml, was given IP at a dose of 1 ml/kg. A 0.9% NaCl was used for control injections in the same volume. Anisomycin (Sigma–Aldrich, USA, Ltd.), was dissolved in HCl (1M), diluted with 0.9% NaCl, to a concentration of 50 μg/μl and the pH was adjusted to 7.4 with NaOH (5 M). The ANI-vehicle was 1 M HC1 diluted in 0.9% sodium chloride, which was adjusted to pH 7.4 with NaOH. Anisomycin and vehicle were administered ICV at a rate of 2 μl/min over 2 min (4 μl/hemisphere) with 1 min given for diffusion, leading to a total dose of 400 μg (200 μg/hemisphere).

All animals from each experiment were given habituation sessions to the infusion procedure prior to the first infusion.

2.8. Histology

At the end of the experiment, rats were sacrificed with a lethal dose of urethane and decapitated. The brains were removed and stored in a 10% Formalin saline solution. Each brain was then frozen, sliced on a freezing microtome at 50 μm and stained with thionin. Cannula placements were confirmed by an investigator blind to the subject's test results. Data from subjects in which a cannula missed the ventricle, or in which ventricles showed signs of infection were discarded.

2.9. Statistical analysis

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

We first examined whether each group developed a significant bias for the drug-paired over the vehicle-paired compartment. The ANOVA (Statistica) was with one repeated measure (the time each animal spent in either compartment). A priori contrasts were chosen since the contrasts of interest were known in advance, and specified by the hypotheses. The α level was set at $p = 0.05$. Finally, an ANOVA comparing the time spent in the left versus 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 anisomycin ICV on consolidation of a morphine-induced place preference

When rats were first given an opportunity to explore the apparatus before training the 43 subjects showed no preference for either compartment ($F = 0.495$, d.f. = 39, $p = 0.688$), confirming the apparatus was unbiased. After one cycle of pairings of drug and vehicle with their respective compartments neither the control group which received vehicle ICV, nor any other group,

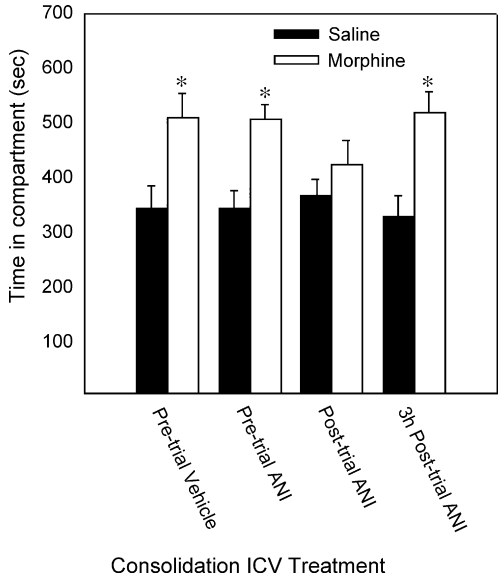

Fig. 1. Effect of anisomycin (ANI) ICV at different infusion times in relation to training on consolidation of a morphine-induced place preference. Data is the time spent in the morphine- and vehicle-paired compartments for the post-consolidation test. * $p < 0.05$ for morphine vs. vehicle-paired.

showed a significant preference for the morphine-paired compartment on the test ($F_s < 4.064$, d.f. = 39, NS), so these data were not analyzed further.

On the second CPP test, after two cycles of training trials, the ICV-vehicle control group showed a significant preference for the drug-paired chamber ($F = 6.565$, d.f. = 39, $p = 0.014$, $N = 11$; Fig. 1). As expected, the group designed to detect any carry over effects of anisomycin infusions (3 h Post-ANI) also showed a strong preference for the drug-paired side ($F = 6.938$, d.f. = 39, $p = 0.012$, $N = 9$). Likewise the group given anisomycin immediately before the training trials (Pre-ANI) showed a significant CPP on test 2 ($F = 6.437$, d.f. = 39, $p = 0.015$, $N = 11$). In contrast, the group given immediate post-training injections of anisomycin (Post-ANI) did not show a preference for the morphine-paired side ($F = 0.866$, d.f. = 39, $p = 0.358$, $N = 12$; see Fig. 1).

A behavioral effect of the anisomycin treatment on the rats was noted. After receiving infusions of anisomycin they were immobile for a few minutes, and moved their heads from side to side. They seemed hyper-reactive to the experimenter's movements and to touch.

3.2. Experiment 2: effect of anisomycin ICV on reconsolidation of a morphine-induced place preference

Before conditioning the 55 subjects showed no bias towards either compartment ($F = 0.085$, d.f. = 51, $p = 0.968$).

Fig. 2A shows the pre-reactivation preferences for each group after two cycles of conditioning trials. The control group to be given vehicle after reconsolidation trials showed a significant preference for the morphine-paired chamber ($F = 16.145$, d.f. = 51, $p = 0.000$, $N = 14$), as did the group to be given anisomycin immediately after reconsolidation trials ($F = 21.484$,

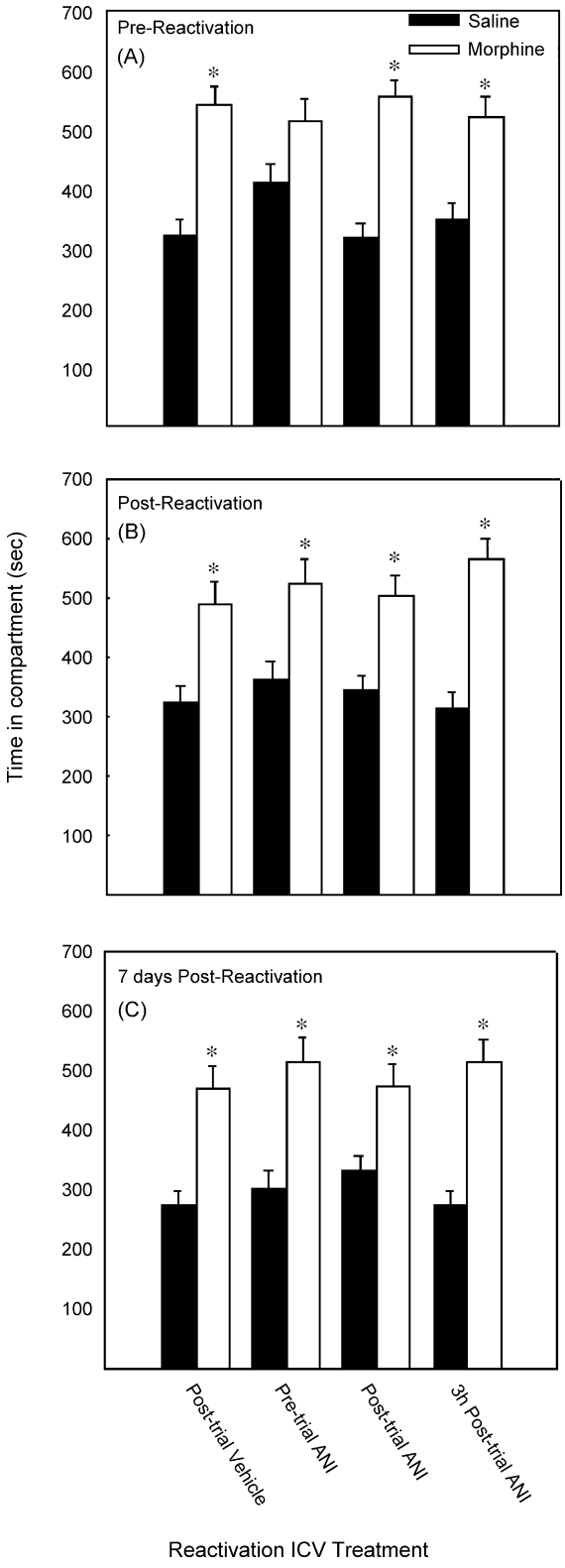

Fig. 2. Morphine-induced conditioned place preference after initial learning (Pre-Reactivation, Panel A), and 48 h after reactivation and ICV treatment (Post-Reactivation, Panel B) and 1 week later (7 day Post-Reactivation, Panel C). Data is time spent in the morphine- and vehicle-paired compartments on each test for groups treated with saline or ANI ICV at different times relative to reactivation. * $p < 0.05$ for morphine vs. vehicle-paired.

d.f. = 51, $p = 0.000$, $N = 16$) and the group to be given anisomycin after 3 h delay ($F = 10.259$, d.f. = 51, $p = 0.002$, $N = 14$). However, for the group to be given anisomycin before reconsolidation trials, the preference for the morphine-paired side did not reach significance ($F = 2.866$, d.f. = 51, $p = 0.097$, $N = 11$). Note this group had a spontaneous bias for the vehicle-paired side of 103.455 seconds before conditioning (NS).

Subjects then experienced another cycle of drug and vehicle pairings to reactivate the memory and were given anisomycin or vehicle ICV in association with the retrieval trials. After one cycle of reconsolidation treatment all four groups showed a significant preference for the morphine-paired chamber (Morphine/Post-vehicle: $F = 8.649$, d.f. = 51, $p = 0.005$; Morphine/Pre-ANI: $F = 12.467$, d.f. = 51, $p = 0.001$; Morphine/Post-ANI: $F = 10.493$, d.f. = 51, $p = 0.002$; Morphine/3 h Post-ANI: $F = 12.229$, d.f. = 51, $p = 0.001$, data not shown). A second cycle of reconsolidation treatments was performed to ensure that any effect was not simply too weak to be detected. It can be seen in Fig. 2B that after the second cycle of reconsolidation treatments all four groups still showed a significant preference for the morphine-paired chamber (Morphine/Post-vehicle: $F = 8.428$, d.f. = 51, $p = 0.005$; Morphine/Pre-ANI: $F = 6.365$, d.f. = 51, $p = 0.015$; Morphine/Post-ANI: $F = 8.980$, d.f. = 51, $p = 0.004$; Morphine/3 h Post-ANI: $F = 19.013$, d.f. = 51, $p = 0.000$). This preference persisted on re-test after 7 days (Morphine/Post-vehicle: $F = 12.617$, d.f. = 51, $p = 0.001$; Morphine/Pre-ANI: $F = 11.504$, d.f. = 51, $p = 0.001$; Morphine/Post-ANI: $F = 7.397$, d.f. = 51, $p = 0.009$; Morphine/3 h Post-ANI: $F = 18.715$, d.f. = 51, $p = 0.000$, Fig. 2C).

As in Experiment 1, it was observed that after receiving infusions of anisomycin rats tended to freeze and exhibited head waving. They were hyper-reactive to the experimenter's movements and to touch.

3.3. Experiment 3: effect of anisomycin ICV on a morphine-induced place preference after a selectively morphine- or saline-paired reactivation

On pre-exposure the 19 subjects showed no spontaneous bias towards either compartment ($F = 0.205$, d.f. = 17, $p = 0.656$). After three cycles of pairings of drug and vehicle with their respective compartments both the group to be reactivated in the morphine-paired compartment (Morphine-paired/ANI: $F = 5.109$, d.f. = 17, $p = 0.037$, $N = 10$) and the group to be reactivated in the saline-paired compartment (Saline-paired/ANI: $F = 6.169$, d.f. = 17, $p = 0.024$, $N = 9$) showed a significant morphine CPP (Fig. 3, Pre-Reactivation).

Forty-eight hours following the single reactivation session, both groups of rats were subjected to a test session. In the group who received anisomycin immediately after a morphine-paired conditioning session, the CPP diminished slightly and was no longer significant (Morphine-paired/ANI: $F = 3.706$, d.f. = 17, $p = 0.071$). In contrast, the group that received anisomycin immediately after the saline-paired reactivation session retained a significant preference for the morphine-paired compartment, which was more reliable than before reactivation

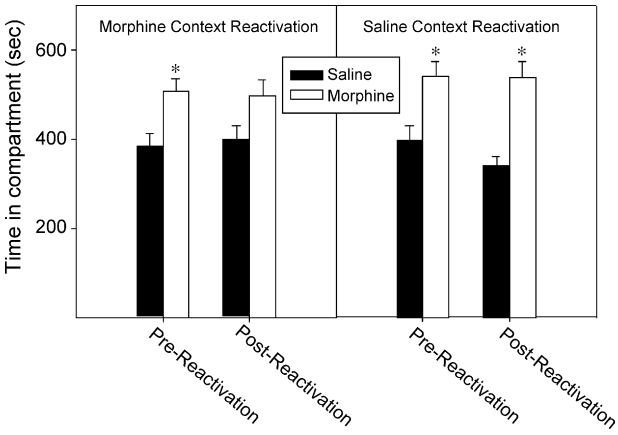

Fig. 3. Data is time spent in the morphine- and vehicle-paired compartments on each test (Pre-Reactivation and Post-Reactivation) for groups treated with ANI ICV following either a morphine-paired or a vehicle-paired reactivation. $^{*}p < 0.05$ for morphine vs. vehicle-paired.

(Saline-paired/ANI: $F = 13.384$, d.f. = 17, $p = 0.002$; Fig. 3, Post-Reactivation).

4. Discussion

Experiment 1 showed that the consolidation of a morphine place preference can be blocked by post-training infusions of anisomycin into the lateral ventricles. Similar infusions given either pre-training, or 3 h post-training did not interfere with the consolidation of the memory for the association of the stimuli in the drug-paired compartment with the rewarding effects of morphine. This confirms that consolidation of a memory for place preference conditioning follows the same requirements for de novo protein synthesis as other types of learning [14,28,29], and shows that ICV anisomycin reaches sites critical for the CPP memory. The results also support previous findings that there is a critical time window during which protein synthesis inhibition needs to occur in order to block memory consolidation [28,30,31], and that the optimal time is the immediate post-conditioning period. It may seem surprising that immediate post-training anisomycin was so effective, given that the training trial last for 20 min and the ICV injection procedure takes several minutes. By comparison in many consolidation paradigms the conditioning phase lasts only a few minutes and consolidation blocking treatments work best if they are applied immediately after training [32–34]. However, training trials are much longer in appetitive paradigms than fear conditioning paradigms, and delayed amnestic treatments have been found to be effective up to 30 min after conditioning [35].

Although our observations of the rats' behavior following ICV anisomycin suggest that anisomycin had direct behavioral effects in addition to protein synthesis inhibition, the fact that the post-training-ANI group failed to develop a CPP cannot be explained by conditioning of aversive effects of the amnestic treatment. There are two arguments against this possibility. First, the anisomycin was given in association with both conditioning compartments. Since reinforcing and aversive influences are additive [36,37], the animal chooses between box cues hav-

ing acquired negative valence on one side, with box cues that have acquired negative valence + positive valence on the other side. Any conditioned aversive effects, therefore, should have little effect on the preference. Secondly, if conditioning of aversive effects to compartment cues reduced the CPP, pre-training anisomycin should have been more effective than post-training anisomycin. With pre-training anisomycin the drug's effects occur in conjunction with both the effects of morphine and the compartment cues, but with post-training amnestic treatment, anisomycin and compartment cues are not associated and associations with morphine effects are long delayed.

Experiment 2 mimicked the protocol of Experiment 1, but this time anisomycin was administered during reactivation sessions after the initial consolidation of morphine context associations had occurred. Even though one group had not exhibited a significant CPP by the end of the initial conditioning, after the two cycles of reconsolidation-blocking treatment, all four groups showed a significant preference for the drug-paired compartment. This preference was retained on the 7 day re-test. Thus, our findings suggest that the memory for a morphine conditioned place preference is not susceptible to disruption by protein synthesis inhibition after reactivation. This result is supported by similar findings in another type of appetitive paradigm. Hernandez et al. [24,33] found that infusions of anisomycin into the nucleus accumbens blocked the initial consolidation of lever-pressing for food in rats, and that anisomycin given systemically after reactivation of lever-pressing behavior impaired performance on subsequent test sessions. However, they showed this latter effect to be the result of a conditioned taste aversion to low doses of anisomycin (5 or 20 mg/kg). Even high doses (150 and 210 mg/kg) of anisomycin known to have previously produced amnesia did not affect the memory other than by taste aversion. They concluded that a well-learned instrumental task did not require protein synthesis-dependent reconsolidation as a means of long-term maintenance, although this may only be true for infusions into the nucleus accumbens.

It is unlikely that the failure to find reconsolidation in these experiments is due to inadequate amnestic treatment, since a dose of 400 μg anisomycin ICV was adequate to block consolidation. It is possible that the biochemistry of consolidation and reconsolidation are not the same. BDNF is required for consolidation but not reconsolidation, and the inverse is true for Zif268, [38]. However, all known mechanisms depend on protein synthesis, and should be susceptible to anisomycin. The possibility that reconsolidation and consolidation take place at different sites, and the reconsolidation site is inaccessible to ICV anisomycin cannot be eliminated. However, previous studies of ICV anisomycin blocking memory in tasks dependent on various structures indicate that ICV anisomycin reaches putative sites such as the frontal cortex, hippocampus and amygdala [26,39–41].

The possibility that our failure to block reconsolidation is attributable to a lack of appropriate reactivation must also be considered. In the CPP paradigm, several possibilities for reactivation can be envisaged. One possibility is to administer anisomycin following a conventional test session. Although this would constitute an extinction trial and possibly weaken the drug-induced preference, Lien et al. have shown that conditioned place preference memory is robust to repeated extinction trials [42]. However, morphine acts as a discriminative stimulus as well as a reinforcer [43] and this drug cue would be absent on a conventional test trial. Since it has been shown that the extent of the amnesia induced by anisomycin depends on how similar the reactivation context is to the original training context [11,44], we reasoned that the most complete reactivation would be to apply the amnestic treatment after both morphine and vehicle conditioning trials. This procedure also has the advantage that it eliminates the possibility of conditioning aversive effects of the amnestic treatment to one compartment.

Experiment 3 was designed specifically to both replicate the method found in recent reports of reconsolidation effects in the CPP [18,21], and to highlight any influence of motivational effects caused by the amnestic treatment. The results were consistent with the possibility that conditioning of aversive effects to the morphine-associated cues may contribute to disruption of a CPP when an amnestic treatment is given after reactivation in the presence of the conditioning drug. In experiment 3, selective association of anisomycin with morphine and the morphine-conditioned context procedure reduced the reliability of a morphine CPP. In contrast, giving the same amnestic treatment after reactivation with vehicle-paired cues in the presence of vehicle seemed to strengthen the preference for the drug-paired cues. These results display a trend which is consistent with conditioning of aversive effects to the context of reactivation. Although these results are predicted from the findings by Hernandez and Kelley, who demonstrated that apparent reconsolidation effects could be the result of taste aversion due to anisomycin [24], the extent to which anisomycin is aversive in a way that may affect place preference is still a matter of debate. A recent study by Kuo et al. report no aversive effects of anisomycin in a place preference paradigm [45]. However, these findings were established in mice, with peripheral injections of anisomycin given 30 min pre-trial. It is likely that the use of a different species and route of injection would affect the level of toxicity caused by anisomycin, and the 30 min delay between the injection and the presentation of the compartmental cues would reduce the chance of any association being acquired. In addition, our study couples a high dose of anisomycin with injections of morphine, which itself produces a conditioned taste aversion [46]. It is possible that these drugs might synergise to produce dysphoria.

The failure to find evidence for reconsolidation effects with positively reinforced associations such as appetitive lever-pressing and place conditioning is open to a number of interpretations. One possibility is that the conditions necessary to demonstrate appetitive learning militate against demonstrating reconsolidation. Most appetitive tasks require multiple training trials, usually spread over several days, and these conditions might be expected to optimize consolidation. Indeed it has been demonstrated with single trial inhibitory avoidance conditioning that vulnerability of a memory to reconsolidation decreases over 1–2 weeks [47]. Findings by Kida and co-workers support the view that stronger memories caused by repeated CS-US pairings are less susceptible to reconsolidation block by anisomycin [48]. Recent findings by Wang et al. [49] suggest

that strong memories may require longer training to reactivation intervals before they undergo reconsolidation and show impairment as a result of anisomycin treatment. It is possible that each consolidation or reconsolidation occasion adds to the strength of the memory, or, if reconsolidation processes are inhibited, reduces the strength of the memory. However, the demonstration of these effects is only possible when the memory is near threshold strength for recall. Once the memory is well above threshold strength, decrements produced by unreconsolidated recall may not bring the memory back to threshold, and remain undetectable. Our procedure used two episodes of reconsolidation blocking treatments for each component of the memory (drug side and vehicle side), but even that seems to have been inadequate to eliminate a well-established memory for drug effects. The anisomycin treatment was strong enough to induce signs of toxicity so that it is probably not possible to further increase the degree of protein synthesis inhibition.

In summary, our results show that consolidation of a morphine-induced place preference can be prevented by post-training ICV infusions of anisomycin, and similar infusions of anisomycin produce an apparent block of reconsolidation when selectively paired with morphine and its associated context during reactivation. However, this effect is no longer present when anisomycin is delivered in a counterbalanced fashion following separate reactivations of both contexts and their associated conditioning treatments (morphine and saline).

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