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Short communication

Dopamine D1 receptors are not critical for opiate reward but can mediate opiate memory retrieval in a state-dependent manner

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HIGHLIGHTS

- D1 receptor homozygous knockout mice demonstrate normal morphine place preferences.
- Blocking rat basolateral amygdala (BLA) D1 receptors during training inhibits this preference.
- Blocking BLA D1 receptors during *both* training *and* testing restores this preference.
- This suggests that BLA D1 receptors mediate state-dependent memory retrieval.

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ABSTRACT

Although D1 receptor knockout mice demonstrate normal morphine place preferences, antagonism of basolateral amygdala (BLA) D1 receptors only during drug-naive rat conditioning has been reported to inhibit the expression of a morphine place preference. One possible explanation for this result is state-dependent learning. That is, the omission of the intra-BLA infusion cue during testing — which acts as a potent discriminative stimulus — may have prevented the recall of a morphine–environment association and therefore, the consequent expression of a morphine place preference. To examine this possibility, we tested whether intra-BLA infusion of the D1-receptor antagonist SCH23390 during both training and testing might reveal a morphine place preference. Our results suggest that in previously drug-naive animals, D1 receptor antagonism during testing restores the opiate conditioned place preference that is normally absent when D1 receptors are blocked only during training, suggesting that BLA D1 receptors can mediate state-dependent memory retrieval.

1. Introduction

Pharmacological antagonism of D1 receptors suggests that they play an important role in opiate reward [1,2], although this result is not supported by work with D1 receptor homozygous knockout mice [3]. Recently, it has been suggested that basolateral amygdala (BLA) dopamine receptors mediate an opiate addiction switch [4]. In previously opiate-naive rats, antagonism of BLA D1 receptors during the training phase of the experiment prevented the expression of a morphine place preference. However, in opiate dependent and withdrawn subjects, antagonism of BLA D1 receptors during training no longer had any effect and instead, BLA D2 receptor blockade now prevented the expression of a morphine place preference. It was hypothesized that these dopamine receptors are critical substrates that enhance emotional salience and the acquisition of opiate reward memories [5].

One possible alternative explanation of these data involves state dependency [6–10]. That is, since intra-BLA antagonist infusions occurred only during training and *not* testing [4], it is conceivable that during testing the subjects were missing a potent discriminative cue necessary for recalling the association between the test environment and the previous morphine administration. This state-dependent memory-cuing mechanism might therefore explain the effect of intra-BLA dopamine receptor antagonism on opiate place preferences.

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To examine this, we first examined whether drug-naive D1 receptor knockout mice would demonstrate a morphine place preference. We predicted that an active D1 receptor was unnecessary for the demonstration of a strong morphine place preference. Additionally, we infused the D1 receptor antagonist SCH23390 into the BLA of previously opiate-naive rats during both training *and* testing, utilizing the same drug doses and conditioning paradigm as a previous study [4]. We hypothesized that subjects that were both trained and tested under the same conditions (i.e., while receiving intra-BLA D1 receptor antagonist infusions) would still acquire and retrieve morphine reward memories, in contrast to those that were only trained under the influence of the antagonist.

14 male or female D1-receptor wild type and homozygous knockout mice (backcrossed to a C57Bl/6 mouse strain for at least 12 generations) and 36 male Wistar rats (Charles River; 350-450 g) were utilized for all experiments at the University of Toronto. Mice were group-housed (3-4 per cage) and rats were singly-housed in Plexiglas cages (22° C, lights on 7:00 a.m. to 7:00 p.m.). Access to standard rodent chow was *ad libitum*. Water was always freely available. All experiments were approved by the University of Toronto Animal Care Committee in accordance with the Canadian Council on Animal Care guidelines.

Mouse conditioning occurred in one of two distinct environments that differed in color and texture (Med Associates Inc., SOF-700RA-25 Two Chamber Place Preference Apparatus, VT, USA). One environment was black with a metal rod floor and the other, white with a wire mesh floor. An intermediate grey area housed a removable partition.

Mice received eight conditioning trials (four alternating morphine and saline vehicle pairings) on separate days. After an *i.p.* injection of 10 mg/kg morphine or saline, they were exposed to one of the conditioning environments for 15 min. All conditioning was unbiased and fully-counterbalanced for treatment compartment and order of drug presentation and there are no baseline preferences for any environment [11]. No locomotor differences were observed between D1 wild type and knockout mice.

After the final conditioning trial, mice were allowed to rest uninterrupted in their home cage for one week until test day. On test day, under drug-free conditions, the mice were allowed to freely explore all three environments (morphine-paired, saline-paired, and neutral) simultaneously by removing the shared partition and introducing the animal into the intermediate grey area separating the two conditioning environments. The time spent in all three compartments was recorded for 10 min.

Rat cannula surgery was performed under isoflurane anesthesia (5% induction, 1-3% maintenance). Ketoprofen (5 mg/kg) was administered as an analgesic. 22-gauge stainless steel guide cannulae (Plastics One, Roanoke, VA, USA) were implanted bilaterally into the BLA using the following coordinates relative to bregma: AP, −3.0 mm; ML, ±5.0 mm; DV, −8.0 mm from the dural surface. Rats were allowed to recover for at least one week prior to conditioning.

SCH23390 (1 μg/0.5 μL) (Sigma) was infused into the BLA bilaterally (0.5 μL per hemisphere). This dose was chosen in accordance with previous studies [4]. Morphine sulphate (5 or 10 mg/kg *i.p.* for rats or mice, respectively) (Almat Pharmachem Inc., Concord, Canada) was dissolved in a 0.9% saline solution.

Rat conditioning took place in one of two distinct environments that differed in colour, texture and smell. One environment (41 cm × 41 cm × 38 cm) was black with a smooth black Plexiglas floor and was scented with 0.3 mL of a 10% acetic acid solution prior to each conditioning session. The other environment had identical dimensions and was white with a metallic mesh floor.

Rats were conditioned using an unbiased, fully-counterbalanced place conditioning procedure. All groups underwent one conditioning session (40 min) per day during the light cycle until a total of eight sessions (four morphine and saline pairings) were completed

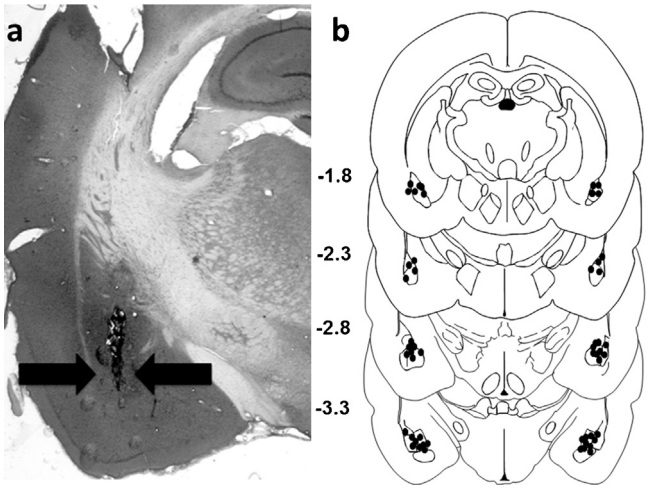

Fig. 1. Basolateral amygdala cannula placements. (a) Photomicrograph showing a representative intra-BLA injector tip. (b) Schematic representation of intra-BLA cannula placements (black circles). Numbers indicate mm caudal from bregma.

(Supplementary Fig. S1). Intra-cranial infusions of SCH23390 or its saline vehicle occurred prior to all conditioning sessions over one minute, plus an additional minute to allow for drug diffusion from the injector tip. Injections of saline or morphine (*i.p.*), and exposure to the respective conditioning environment followed.

Testing occurred one week after conditioning was completed. Rats received intra-BLA infusions of SCH23390 or its saline vehicle prior to placement on a neutral grey zone (41 cm × 10 cm) separating the two conditioning environments. Each subject was allowed to freely explore all three distinct areas over a 10-min period. The time spent in all three compartments was recorded using EthoVision XT (version 5) software (Noldus Information Technology, Ottawa, Canada). At the end of the experiments, all rats were classified into one of four distinct experimental groups according to their intra-BLA infusion treatments (vehicle, V or antagonist, A) during training and testing: (1) V/V (control group receiving vehicle during both training and testing) (2) A/V (antagonist only during training, vehicle during testing) (3) A/A (antagonist during both training and testing) (4) V/A (vehicle during training, antagonist only during testing). Intra-BLA SCH23390 infusions did not have any observable locomotor or behavioural effects.

After testing, rats were deeply anesthetized with sodium pentobarbital (0.8 mg/ml, *i.p.*; drug obtained from Animal Resources Centre, Montreal, Canada) and perfused transcardially with 200 mL each of saline and 4% formaldehyde. Brains were rapidly removed and stored for 24 h in a 25% sucrose/4% formaldehyde post-fixative, sliced into 30-μm-thick sections, and mounted on gelatin-coated slides. Correct placements were verified with cresyl violet staining and light microscopy [12]. Investigators were blind to the behavioural performance of the animals during analyses. Subjects were excluded if any placements were situated outside of the BLA. A representative photomicrograph and a schematic of BLA cannula placements are shown in Fig. 1. Data were analyzed using analysis of variance (ANOVA) and Tukey HSD *post hoc* test where applicable (alpha = 0.05).

Using previously drug-naive, non-deprived male and female D1 wild type and homozygous knockout mice, we examined the motivational effects of 10 mg/kg morphine. A 3-way ANOVA [with gender and genotype as between-group factors and conditioning drug (saline vs. morphine) as a within-group factor] revealed a significant main effect of conditioning drug [$F_{(1,10)} = 31.3$, $P = 0.0002$] but no other significant main effects or interactions, indicating that the D1 wild type and homozygous knockout groups were not significantly different from one another (Fig. 2). As no difference was

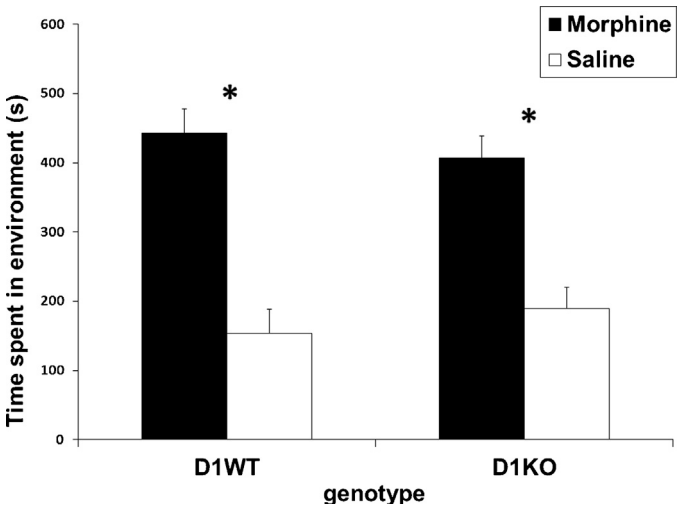

Fig. 2. The effect of D1 receptors on morphine motivation. Opiate-naive D1 wild type and knockout mice both demonstrated strong morphine place preferences (*$P < 0.05$) for an environment paired with 10 mg/kg morphine (black bars). Data represent means ± SEMs of time spent in each environment. Since no differences were observed between males and females, their data were pooled.

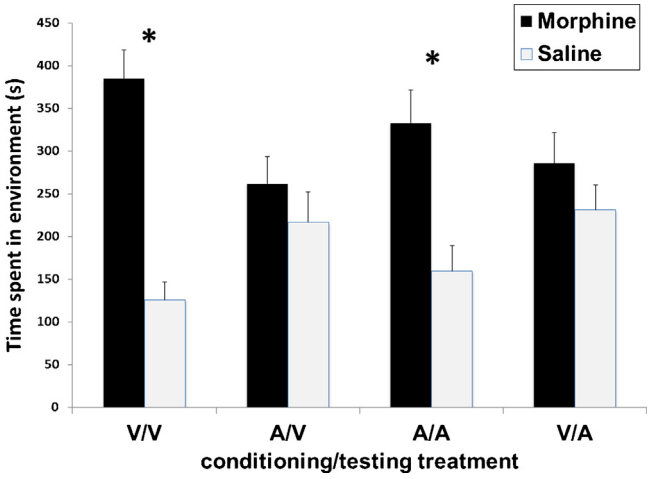

Fig. 3. The effect of intra-BLA D1 receptor blockade on the acquisition and recall of a morphine place preference. Opiate-naive rats were divided into four treatment groups based on their intra-BLA drug infusions (vehicle or 1.0 μg/0.5 μL of the D1 receptor antagonist SCH23390) during training and testing: V (vehicle)/V, A (antagonist)/V, A/A, and V/A. In the A/V and V/A groups where training and testing infusions did not match, no morphine place preferences were observed. Conversely, the V/V and A/A groups showed significant place preferences (*$P < 0.05$) for an environment paired with 5 mg/kg morphine (black bars). Data represent means ± SEMs of time spent in each environment.

observed between male and female mice, their data were combined.

Using previously drug-naive, non-deprived rats, we performed intra-BLA infusions of vehicle (V) or SCH23390 (A, antagonist) during training/testing. Consequently, there were four experimental groups: V/V (control) ($n = 11$), A/V (antagonist only during training) ($n = 9$), A/A (antagonist during both training and testing) ($n = 8$), and V/A (antagonist only during testing) ($n = 8$). A 3-way ANOVA [with training infusion (vehicle vs. SCH23390) and testing condition (identical vs. different) as between-group factors and conditioning drug (saline vs. morphine) as a within-group factor] revealed a significant main effect of conditioning drug [$F_{(1,32)} = 16.8$, $P = 0.0003$] and a significant interaction between training infusion and testing condition [$F_{(1,32)} = 6.59$, $P = 0.015$] (Fig. 3). There were no other significant main effects or interactions. A post hoc Tukey HSD test revealed that only in the V/V and A/A groups (and not the V/A or A/V groups) did morphine produce a significant place preference ($P < 0.05$). The V/V and A/A groups were not significantly different from each other. The cannula placements for the various groups are shown in Fig. 1.

Our findings replicate the work of Lintas et al., who demonstrated that intra-BLA infusion of a D1 receptor antagonist during the acquisition phase of a morphine place preference paradigm was sufficient to prevent the expression of that place preference during testing [4]. However, our results argue that BLA D1 receptor activity is unnecessary for the demonstration of a morphine place preference as both D1 receptor knockout mice and rats administered intra-BLA SCH23390 during conditioning and testing were able to display morphine place preferences. Only when intra-BLA SCH23390 administration was present during one of training or testing (but not both), was a block of morphine place conditioning observed. It remains an open question as to where in the brain morphine place preferences are, in fact, learned. Our work suggests that, wherever this learning is occurring, BLA D1 receptors are not crucial for this process, at least in drug-naive animals.

As test-day intra-BLA SCH23390 was able to reveal a morphine place preference in rats that received intra-BLA SCH23390 during conditioning, this suggests that BLA D1 receptor antagonism has no effect on reward or memory acquisition. Although previous work has suggested that *increased* activity at these receptors increases the salience of a sub-threshold dose of morphine [5], the present data suggest that a *reduction* in activity can also reveal opiate reward. One explanation of the retrieval ability of test-day BLA SCH23390 is its ability to function as a state-dependent memory cue in drug-naive (but not drug-dependent) animals [7,8].

Mechanisms that prevent the formation and/or retrieval of drug-related reward memories have been investigated as a means to combat addiction [13–15]. Two major theories concerning this idea are the motivational deficit model [16] and the memory cuing model [17,18]. The former posits that rewards that lack sufficient emotional salience prevent subjects from either experiencing the reward or from forming appropriate reward memories. Conversely, the latter states that a subject's inability to recall a reward memory is due to the absence of critical cues required to trigger the retrieval process (e.g., environmental cues or neurological states). Our findings support this second interpretation, as BLA D1 receptor activity is unnecessary for the rewarding effects of morphine in drug-naive animals. This result is consistent with the observed lack of effect of D1 receptor knockouts on morphine place preferences, reported both here and elsewhere [3]. In these cases, training and testing occur under identical conditions, i.e., D1 receptor activity is always lacking.

Our work contrasts with previous reports that pharmacological antagonism of D1 receptors (during conditioning only) blocks morphine reward [1,2]. We propose that intra-BLA SCH23390 infusions serve as potent retrieval cues for previously-formed opiate reward memories, but only if these memories were formed in the presence of SCH23390 [7]. That is, a morphine-environment association formed in the presence of intra-BLA SCH23390 is only successfully recalled if SCH23390 is also present during testing, as if the antagonist and conditioning environment have been combined into a compound cue. Indeed, test-day intra-BLA SCH23390 *blocked* morphine place preferences in rats that only received vehicle infusions during conditioning (group V/A). We suggest that a state-dependent model of memory cuing can explain intra-BLA D1 receptor antagonist-induced deficits in reward memory retrieval.

The precise nature of this discriminatory cue remains unclear. For example, the administration of another drug during the test session might substitute for this effect and also produce the successful retrieval of the previous reward memory. Indeed, research suggests that BLA D1 receptors mediate fear and anxiety [19,20],

states which could be construed as potent discriminative cues in and of themselves — although, for drug-dependent animals, these particular cues no longer appear to be relevant [4]. In any case, we would predict that this cue is not specific for morphine and may extend to other stimuli of interest as well.

These results raise the question as to why intra-BLA D1 receptor antagonism (during training only) blocks the development of opiate conditioned place preferences in opiate-naive rats, whereas systemic (and intra-nucleus accumbens) administration of the broad-spectrum D1 and D2 dopamine receptor antagonist alpha-flupenthixol (during training only) does not [21,22]. Indeed, past work has shown that morphine place preferences in opiate-naive rats are blocked not by dopamine receptor antagonism but rather, by lesions of the brainstem tegmental pedunculopontine nucleus [23]. One possible way to account for this discrepancy between systemic and BLA dopamine receptor blockade is to propose that the latter is a much more distinctive perceptual cue than the former. This could be due to systemic administration of alpha-flupenthixol being unable to sufficiently generate a similarly-effective BLA dose as compared with direct intra-BLA infusion. Alternatively, it is possible that the non-D1 actions of alpha-flupenthixol — e.g., its effects on D2 or $5HT_2$ receptors [24,25] — somehow negate or overshadow its D1 receptor antagonist cuing effect. Last, it is possible that systemic drug administration produces effects in areas outside of the BLA that might counteract its direct actions in the BLA. Future work is needed to adequately examine these possibilities.

In summary, our data extend the results of Lintas et al. [4] and replicate their finding that intra-BLA D1 receptor antagonism during conditioning inhibits the expression of a morphine place preference. However, our work suggests that a state-dependent model of memory cuing can explain intra-BLA D1 antagonist-induced deficits in morphine reward memory retrieval; BLA D1 receptor activity itself, is unnecessary for an opiate place preference. Instead, we argue that BLA D1 receptor blockade has distinct perceptual functions in opiate-naive rats and this distinct discriminative cue must be present during both training and testing — or during neither — for the successful recall and expression of an opiate place preference.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbr.2013.03.026.

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