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Different neural systems mediate morphine reward and its spontaneous withdrawal aversion

Hector Vargas-Perez,[1] Ryan Ting-A-Kee[2] and Derek van der Kooy[1,2]
[1]Department of Molecular Genetics, Donnelly Centre for Cellular & Biomolecular Research, University of Toronto, Toronto, ON M5S 3E1, Canada
[2]Institute of Medical Science, University of Toronto, Canada

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Abstract

The opponent-process theory posits that the aversive state of acute opiate withdrawal is a consequence of, and depends on, the previous rewarding state evoked by acute morphine reward. Although the brainstem tegmental pedunculopontine nucleus (TPP) is crucial for the rewarding component of morphine, the source of the later aversive component is not known. It is possible that (i) the second aversive process takes place within the TPP itself or (ii) morphine reward in the TPP activates an unconditioned opponent motivational process in another region of the brain. The effects of reversible inactivation of the TPP on the motivational properties of acute morphine and its spontaneous withdrawal effects in non-drug-dependent rats were examined using a place-conditioning paradigm. Reversible inactivation of the TPP with lidocaine or bupivacaine immediately before the morphine injection blocked the rewarding properties of morphine in non-dependent rats. Blocking the rewarding effects of morphine also blocked the opponent aversive effects of acute morphine withdrawal. In contrast, reversible inactivation of the TPP during the acute morphine withdrawal did not block this opponent aversive process. Our results confirm that the TPP is a critical neural substrate underlying the acute rewarding effects of morphine in non-dependent rats. Furthermore, the opponent aversive process of acute morphine withdrawal is induced by the acute rewarding effects of morphine. However, the TPP does not directly mediate the spontaneous withdrawal aversion (the opponent process), suggesting that a different system, triggered by the changes in the TPP after the primary drug response, produces the aversion itself.

Introduction

The opponent-process theory of motivation states that any stimulus activates two opposing processes (Solomon & Corbit, 1974). The first process has a fast start and ends quickly, similar in timing to the actual stimulus. The second process is slower to start and slower to end, lasts longer than the stimulus and opposes the actions of the first process (Solomon & Corbit, 1974). The onset of the opponent process can be seen as a manifestation of a homeostatic control mechanism that brings the organism's state back to 'normal' operating levels (Solomon & Corbit, 1974; Solomon, 1980). The opponent-process theory accounts for the dynamics of a diverse array of physical and affective experiences such as the contingent color after-effect (McCollough, 1965; Hurvich, 1981) and drug addiction (Solomon & Corbit, 1973, 1974; Koob *et al.*, 1989a).

The spontaneous withdrawal aversion observed after acute morphine administration can be explained by the opponent-process theory (Koob *et al.*, 1989a; Vargas-Perez *et al.*, 2007). More precisely, in non-dependent rats the administration of the drug produces a rewarding state and then, after the drug has been cleared from the organism, an aversive state emerges that is inextricably linked to the first rewarding state (Fig. 1C). The opponent-process theory has been tested in non-dependent rats using tegmental pedunculopontine nucleus (TPP) lesions. The TPP is a critical region in the neural system that subserves the rewarding effects of drugs, such as opiates, stimulants (i.e. amphetamines) (Bechara & van der Kooy, 1989) and nicotine (Laviolette *et al.*, 2002). TPP lesions selectively remove the acute reward produced by morphine but not other acute effects of morphine such as conditioned taste aversion or analgesia (Vargas-Perez *et al.*, 2007) (Fig. 1A and B). When rats are unable to sense the reward state related to acute morphine administration, the aversive second opponent process does not emerge (Fig. 1D).

The opponent-process theory suggests that the brain contains dynamic control mechanisms that counter or oppose all changes from an equilibrium or basal level of activity (Solomon, 1980; Koob *et al.*, 1989a). In this way, the initial acute rewarding effect of morphine is counteracted by an adaptation in the system that mediates the primary drug response. When morphine leaves the system, the existence of this neural adaptation is the source of the aversive state seen with acute morphine withdrawal (Koob *et al.*, 1989a).

It is intuitively logical to hypothesize that the neural elements responsible for acute drug reward would also be responsible for mediating the aversion associated with the withdrawal. For example, increased activity in a brain region associated with reward could be balanced by a later decrease of activity in that same brain region to

Correspondence: Dr H. Vargas-Perez, as above.
E-mail: vargashector@yahoo.com

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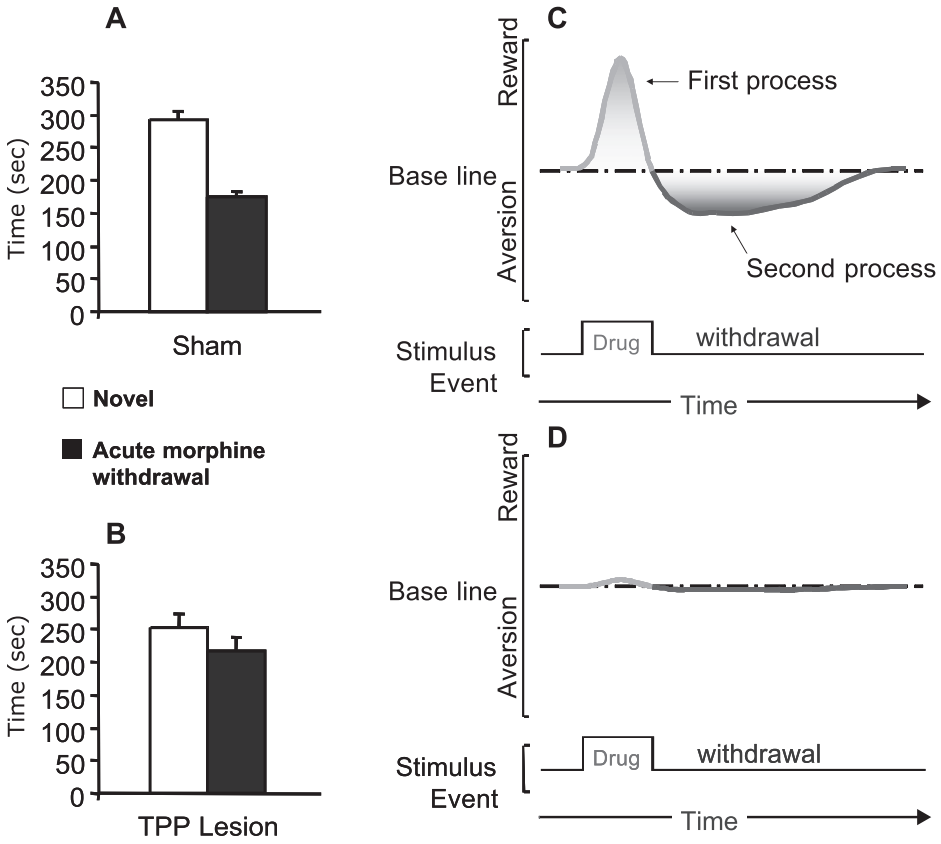

FIG. 1. A test of the opponent-process theory of motivation in non-dependent rats using TPP lesions. (A and B) The graphs (from Vargas-Perez *et al.*, 2007) show the effects of sham or excitotoxic TPP lesions on 16 h abstinence from morphine (20 mg/kg) in non-dependent rats. The data represent the means ± SEM of the absolute time spent in the previously unfamiliar novel and morphine withdrawal-paired compartments. (A) In sham-lesioned rats, abstinence from acute doses of morphine produced robust conditioned place aversions for the spontaneous withdrawal-paired environment. (B) TPP lesions blocked the aversions for places paired with acute morphine withdrawal. (C and D) A model of the opponent-process theory of motivation tested in non-dependent rats using TPP lesions. (C) An acute injection of morphine in non-dependent animals evokes an acute rewarding response (the positive first process) followed by a later aversive response (the opponent process). (D) Because the second opponent process depends on the emergence of the positive first process, blocking the rewarding effects of morphine with TPP lesions also blocked the later acute morphine withdrawal aversive response.

produce a rebound aversion. As the TPP is a critical neural substrate underlying the motivational effects of opiates in non-dependent rats (Bechara & van der Kooy, 1992; Olmstead & Franklin, 1993), it is reasonable to hypothesize that a drug-induced, primary neuronal response element in the TPP would itself adapt to neutralize the acute rewarding effects of morphine.

The persistence of this neural adaptation in the TPP after the morphine clears the organism would be the cause of the aversive withdrawal response. However, it is also feasible that the aversion after acute morphine reward is produced by an adaptation in a different neural system, triggered by the changes in the TPP after the primary drug response. In the present study, a direct role for the TPP in mediating the aversive effects of spontaneous withdrawal from acute morphine administration was assessed in non-dependent rats. Using an unbiased place-conditioning paradigm, we examined the effects of reversible inactivation of the TPP with local anesthetics on the motivational properties of acute morphine and its spontaneous withdrawal effects. Similar to what has been observed with excitotoxic TPP lesions (Bechara & van der Kooy, 1992; Olmstead & Franklin, 1993; Vargas-Perez *et al.*, 2007), reversible inactivation of the TPP during opiate administration blocked the rewarding properties of morphine in non-dependent rats. However, reversible inactivation of the TPP during the spontaneous withdrawal phase did not block the acute morphine withdrawal aversion, suggesting that the TPP does not directly mediate the opponent aversive effect of acute morphine withdrawal.

Materials and methods

Animals and surgical procedures

Male Wistar rats (Charles River; weighing 350–450 g during the experiment) were housed individually in Plexiglas cages in a room maintained at 22 °C and lit from 07:00 to 19:00 h. Rats were given food and water *ad libitum* throughout the experiment. Rats were anesthetized with inhaled isoflurane (5% to induce anesthesia and 2–3% to maintain anesthesia) and placed in a stereotaxic device. For microinfusion cannulae, 22-gauge stainless steel guide cannulae (Plastics One, Roanoke, VA, USA) were bilaterally implanted 2 mm dorsal to the TPP at a 10° angle using the following coordinates relative to bregma: AP, −7.6 mm; ML, ± 3.1 mm and DV, −6.6 mm from the dural surface. At least 2 weeks were allowed for post-surgical recovery preceding behavioral training. At the start of the experiments, all of the animals were drug naive. At the end of experiments, rats were deeply anesthetized with sodium pentobarbital (Somnotol, 0.8 ml/Kg, i.p.) and were perfused transcardially with 200 mL of 0.9% saline followed by 400 mL of 10% formalin. Brains were rapidly removed and stored for 12 h in 25% sucrose in a 10% formalin solution. Brains were then flash frozen at -70°C, sliced in a freezing microtome at -20°C into 40μm-thick sections, and mounted on gelatine-coated slides. Sections of the TPP were processed for cresyl violet staining and subsequently examined by light microscopy. All experiments were approved by the University of Toronto Animal Care Committee, in accordance with the Canadian Council on Animal Care guidelines (http://www.ccac.ca/).

Drug treatments

The drugs used in these experiments were morphine sulfate (Almat Pharmachem Inc.), nicotine hydrogen tartrate salt (Sigma), naloxone hydrochloride (Sigma), lidocaine hydrochloride (Sigma) and bupivacaine hydrochloride (AstraZeneca). Bilateral TPP microinjections of lidocaine (4% in 0.5 μL volume per infusion) or its phosphate-buffered saline (PBS) vehicle were performed over 1 min, just before place conditioning. Bilateral TPP microinjections of bupivacaine

(0.5% in 0.5 μL volume per infusion) vehicle were performed over 1 min, just before morphine administration. Infusion cannulae were left in place for an additional 1 min post-infusion to allow for spread of the drug from the injector tip. All other drugs were administered systemically (morphine and naloxone were administered intra-peritoneally, nicotine was administered subcutaneously).

Place-conditioning apparatus and conditioning

The place-conditioning apparatus was identical to that described previously (Mucha *et al.*, 1982; Bechara & van der Kooy, 1992). Conditioning took place in one of two distinct environments that differed in color, texture and smell, consisting of a black-walled chamber (41 × 41 × 38 cm) with a smooth, black plexiglas floor, wiped with 0.3 mL of 3% acetic acid before each conditioning session, and a white-walled chamber (41 × 41 × 38 cm) with a mesh metallic ?oor. During testing, each rat was placed in a neutral gray zone (41 × 10 cm) that separated the two compartments and was allowed to explore both environments freely for a period of 10 min. Testing was performed drug-free between 48 and 72 h after the final conditioning session.

Two place-conditioning procedures were used in the present set of experiments. For experiments that examined the direct effect of the drug, rats were conditioned with a fully counterbalanced place-conditioning procedure. In this procedure, rats were exposed to both the black and white conditioning environments in a fully counterbalanced order. All experimental groups received four drug-environment and four saline-environment conditioning sessions for 40 min over eight consecutive days. To assess the effect of spontaneous withdrawal from morphine, we employed a modified place-conditioning procedure (Procedure W or withdrawal-paired), during which conditioning took place in only one compartment of the place-conditioning apparatus (Bechara *et al.*, 1992, 1995). This single-side-withdrawal procedure has been shown to measure only the aversive motivational effects of morphine withdrawal, separate from the rewarding value of morphine itself (Bechara *et al.*, 1992, 1995). Furthermore, no significant effects of novelty have been detected on testing with this modified procedure, when saline is paired with one environment and no pairings are given in the other environment during training (Bechara & van der Kooy, 1992; Bechara *et al.*, 1992, 1995). Indeed, in a control group of rats run here, no significant difference was found between the times spent during testing on the previously unfamiliar neutral vs. saline-paired compartments (mean ± SEM: neutral compartment, 186.9 ± 37.485; saline-conditioned compartment, 234.21 ± 45.535; $t_5 = 0.94$, $P > 0.05$).

To measure the aversive motivational effects of morphine withdrawal, each non-dependent rat was given morphine (20 mg/kg, i.p.) and returned immediately to its home cage. Previous work has shown that the time window to observe spontaneous withdrawal aversions in non-dependent rats is between 11 and 16 h after the last injection of morphine, as both shorter (3.5 h) and longer (24 h) intervals do not produce motivational effects (Bechara *et al.*, 1995). Therefore, at approximately 16 h after morphine injection, each rat was injected with saline vehicle and then confined immediately to a distinct conditioning environment for 40 min. This procedure was repeated four times over 8 days. In this way, one of the two compartments was paired with the absence of morphine and the other was an unfamiliar, neutral environment at testing. Designation of the conditioning compartments was counterbalanced. All data were analysed by two-tailed paired Student's *t*-tests.

Results

Effect of reversible inactivation of tegmental pedunculopontine nucleus on the motivational properties of morphine

All TPP-cannulated rats included in statistical analyses were verified histologically to confirm that placements of the cannulae were in the TPP. Ten of 88 rats with cannulae aimed at the TPP were excluded following histological analysis. Figure 2C shows a photomicrograph of representative TPP cannulae.

Using an unbiased place-conditioning procedure, we found that when lidocaine was infused into the TPP immediately prior to conditioning the rewarding properties of systemic morphine (20 mg/kg, i.p.) were blocked (Fig. 2A). There was a significant difference between the times spent in the previously morphine vs. saline vehicle-paired compartments in intra-TPP PBS-infused rats ($t_7 = 5.13$, $P < 0.05$) ($n = 8$) but not when intra-TPP lidocaine was infused ($t_7 = 0.56$, $P > 0.05$) ($n = 8$). In a separate group of rats ($n = 10$), intra-TPP lidocaine infusion proved to have no motivational effects of its own (Fig. 2B). There was no significant difference between the times spent during testing in the previously intra-TPP lidocaine vs. saline vehicle-paired compartments ($t_9 = 1.38$, $P > 0.05$) ($n = 10$).

Intra-tegmental pedunculopontine nucleus lidocaine infusions do not impair place-conditioning learning

To test for possible learning impairments induced by reversible TPP inactivation, we tested intra-TPP lidocaine-infused rats in the place-conditioning paradigm with nicotine. The acute aversive properties of nicotine (0.8 mg/kg) in place conditioning were not blocked by intra-TPP lidocaine (Fig. 2D). There was a significant difference between the times spent during testing in the previously nicotine vs. saline vehicle-paired compartments in both intra-TPP PBS ($t_7 = 5.04$, $P < 0.05$) ($n = 8$) and intra-TPP lidocaine ($t_7 = 3.15$, $P < 0.05$) ($n = 8$) rats.

Intra-tegmental pedunculopontine nucleus lidocaine infusion blocks naloxone aversion

In order to test if the aversive acute morphine withdrawal state in non-dependent rats has similarities to the aversive pharmacological effects of opioids being displaced from opiate receptors, we tested intra-TPP lidocaine-infused rats in the place-conditioning paradigm with the opiate receptor antagonist naloxone (5.0 mg/kg, i.p.). We found that reversible TPP inactivation did in fact block the acute aversive properties of naloxone (Fig. 2E). There was a significant difference between the times spent during testing in the previously naloxone vs. saline vehicle-paired compartments in intra-TPP PBS- ($t_7 = 2.96$, $P < 0.05$) ($n = 8$) but not in intra-TPP lidocaine-infused ($t_{10} = 0.24$, $P > 0.05$) ($n = 11$) rats.

The aversive effects of acute morphine withdrawal are not blocked by reversible tegmental pedunculopontine nucleus inactivation at the time of the withdrawal

In rats infused with PBS into the TPP while 16 h abstinent from morphine (20 mg/kg), a robust conditioned place aversion for the spontaneous withdrawal-paired environment was observed. Similarly, rats infused with intra-TPP lidocaine during acute morphine withdrawal also exhibited a conditioned place aversion for the withdrawal-paired compartment (Fig. 3A). Significant differences between the times spent during testing on the previously unfamiliar neutral vs. withdrawal-paired compartments were seen in both the PBS- ($t_7 = 2.55$, $P < 0.05$) ($n = 8$) and lidocaine-treated ($t_8 = 3.89$, $P < 0.05$) ($n = 9$) rats. Thus,

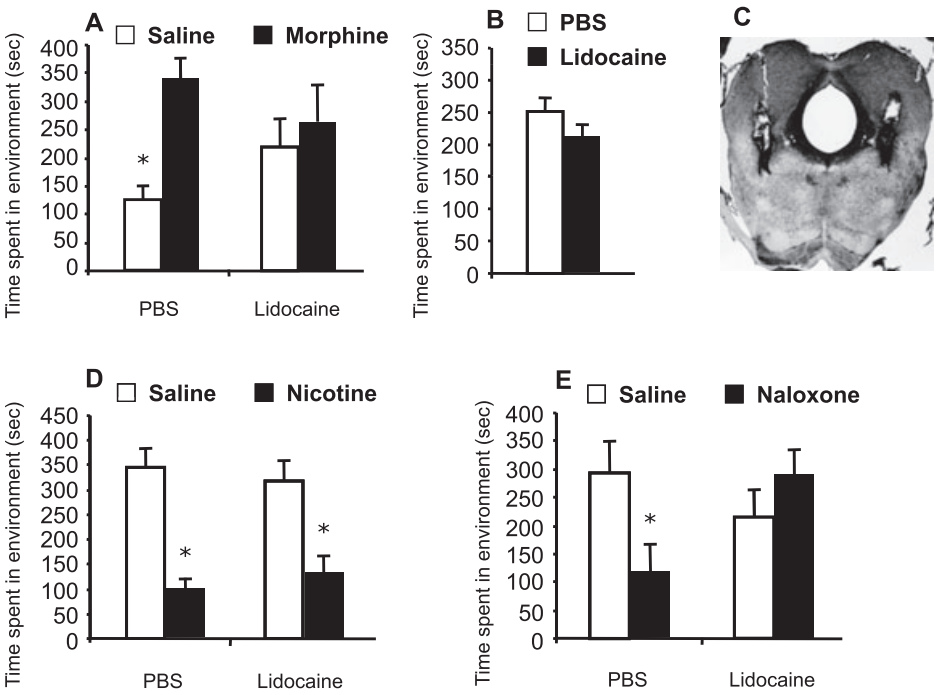

FIG. 2. The effect of reversible inactivation of the TPP on motivated behaviors in non-dependent rats. (A) Intra-TPP lidocaine (4%) infusions blocked the morphine-conditioned place preference. Data represent means ± SEM of the absolute times spent during testing in the previously saline and previously morphine (20 mg/kg) paired compartment (*$P < 0.05$) (PBS, $n = 8$; lidocaine, $n = 8$). (B) Intra-TPP lidocaine infusions do not cause any aversive or rewarding effects of their own. Data represent means ± SEM of the absolute times spent in the previously PBS and previously lidocaine paired compartments ($n = 10$). (C) Cresyl violet-stained coronal section of a typical bilateral intra-TPP cannulae placement (10 × magnification). (D) Intra-TPP lidocaine infusions did not block conditioned place aversions induced by nicotine (0.8 mg/kg). Data represent the means ± SEM of the absolute times spent in the previously saline and previously nicotine paired compartments (*$P < 0.05$) (PBS, $n = 8$; lidocaine, $n = 8$). (E) Naloxone-conditioned place aversions were blocked by intra-TPP lidocaine infusions. Data represent the means ± SEM of the absolute times spent in the previously saline and previously naloxone (5 mg/kg) paired compartments (*$P < 0.05$) (PBS, $n = 8$; lidocaine, $n = 11$).

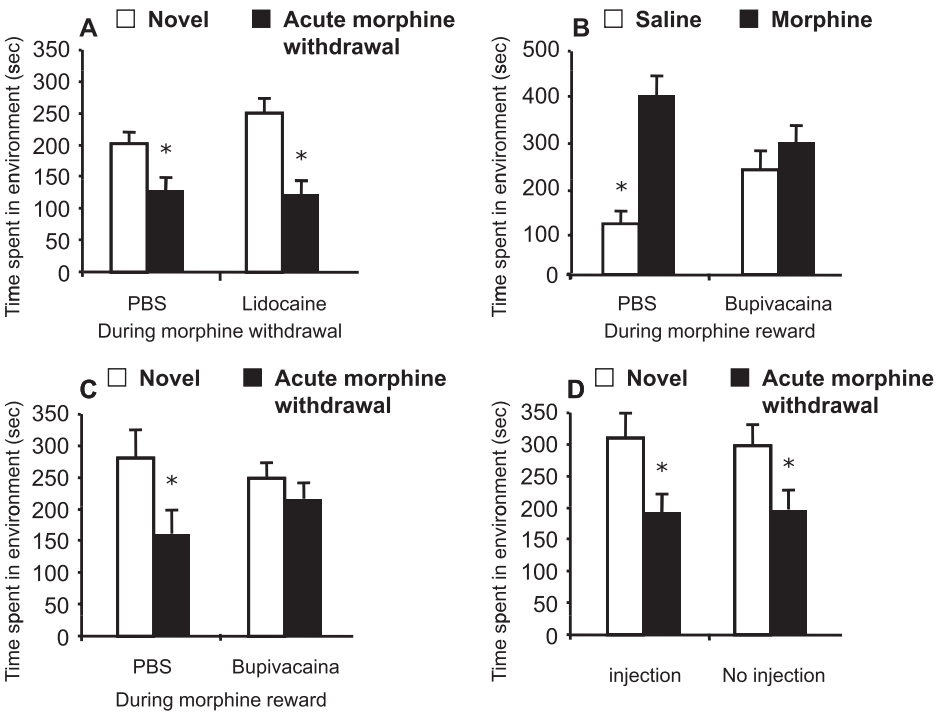

FIG. 3. (A) Intra-TPP lidocaine (4%) infusions did not block the aversions for compartments paired with 16 h abstinence from acute doses of morphine (20 mg/kg) in non-dependent rats. Data represent the means ± SEM of the absolute times spent in the previously unfamiliar novel and morphine withdrawal-paired compartments (*$P < 0.05$) (PBS, $n = 8$; lidocaine, $n = 9$). (B) Intra-TPP bupivacaine infusions also blocked the acute morphine-conditioned place preference. Data represent means ± SEM of the absolute times spent during testing in the previously saline and previously morphine (20 mg/kg) paired compartment (*$P < 0.05$) (PBS, $n = 8$; bupivacaine, $n = 7$). (C) Intra-TPP bupivacaine (0.5%) infusions during morphine reward blocked the aversions for compartments paired with 16 h abstinence from acute doses of morphine (20 mg/kg) in non-dependent rats. Data represent the means ± SEM of the absolute times spent in the previously unfamiliar novel and morphine withdrawal-paired compartments (*$P < 0.05$) (PBS, $n = 7$; lidocaine, $n = 7$). (D) The lack of injection with saline vehicle before conditioning trials did not block the aversions for compartments paired with 16 h abstinence from acute doses of morphine (20 mg/kg) in non-dependent rats. Data represent the means ± SEM of the absolute times spent in the previously unfamiliar novel and morphine withdrawal-paired compartments (*$P < 0.05$) (injected, $n = 7$; not injected, $n = 9$).

both TPP PBS- and lidocaine-treated rats showed significant aversions for places paired with acute morphine withdrawal.

Tegmental pedunculopontine nucleus inactivation during morphine reward blocks the aversive effects of acute morphine withdrawal

Blocking the rewarding effects of morphine with reversible inactivation of the TPP also blocked the opponent aversive effects of acute morphine withdrawal (Fig. 3C). To block the entire time-course of morphine reward and not just the short time that the animals were in the place-conditioning apparatus immediately after morphine or saline injection, the TPP was reversibly inactivated with the local anesthetic bupivacaine, which has an effect that lasts for at least 2 h (Swerdlow & Jones, 1970; Covino, 1986), compared with the time-course (approximately 30–60 min) of the faster acting anesthetic lidocaine (Covino, 1986). A significant difference between the times spent during testing on the unfamiliar neutral vs. withdrawal-paired compartments was seen in intra-TPP PBS-infused rats ($t_6 = 2.198$, $P < 0.05$) ($n = 7$) but not in intra-TPP bupivacaine-infused rats ($t_6 = 0.937$, $P > 0.05$) ($n = 7$). Thus, intra-TPP PBS, but not intra-TPP bupivacaine, rats showed significant aversions for places paired with acute morphine withdrawal.

Similar to lidocaine, infusing bupivacaine into the TPP immediately prior to conditioning also blocked the acute rewarding properties of systemic morphine (20 mg/kg, i.p.) (Fig. 3B). There was a significant difference between the times spent in the previously morphine vs. saline vehicle-paired compartments in intra-TPP PBS-infused rats ($t_7 = 5.13$, $P < 0.05$) ($n = 8$) but not when intra-TPP bupivacaine rats ($t_6 = 0.98$, $P > 0.05$) ($n = 7$) were infused.

It is possible that the aversion seen in morphine-withdrawn animals partly reflects the pain generated by the needle of the vehicle injection procedure before conditioning trials, due to a long-lasting increase in pain sensitivity (Laulin *et al.*, 1998, 1999). To control for this, a group of rats were conditioned after 16 h of morphine withdrawal without vehicle injections. Both vehicle-injected ($t_6 = 2.41$, $P < 0.05$) ($n = 7$) and non-injected ($t_7 = 2.26$, $P < 0.05$) ($n = 8$) rats showed equivalent and significant aversions for places paired with acute morphine withdrawal (Fig. 3D). Thus, the aversion to morphine withdrawal is not due to an increase in pain sensitivity.

Discussion

The opponent-process theory of motivation posits that, as a consequence of a primary affective process triggered by an arousing stimulus, a second process that counters the departure from a state of equilibrium is evoked (Solomon & Corbit, 1974). Spontaneous withdrawal aversion is the second process that works to counteract acute morphine reward (Koob *et al.*, 1989a; Vargas-Perez *et al.*, 2007) and is not just a long-lasting increase in pain sensitivity. When the rewarding effects of morphine are blocked, as they are with TPP lesions (Vargas-Perez *et al.*, 2007) or with reversible inactivation of the TPP with bupivacaine, then the aversive second opponent process does not emerge. This suggests that the acute rewarding effects of morphine induce the opponent aversive process of acute morphine withdrawal. Therefore, the TPP could be thought of as playing a key role in at least initiating the spontaneous withdrawal aversion. We tested whether the reversible inactivation of the TPP, after morphine has produced its acute rewarding effects, would block the acute morphine withdrawal aversion, i.e. is the aversive second process occurring in the TPP itself? The present results suggest that, although the TPP initiates the opponent process, the opponent aversive process itself does not take place in the TPP. Thus, a fundamentally distinct neural system mediates acute morphine reward vs. spontaneous morphine withdrawal aversion in non-dependent rats.

As with excitotoxic TPP lesions (Bechara & van der Kooy, 1992; Olmstead & Franklin, 1993; Vargas-Perez *et al.*, 2007), reversible TPP inactivation blocked the acute rewarding effects of morphine administration. It is possible that this reversible TPP inactivation is producing a motivational state that overshadows the rewarding properties of morphine. However, intra-TPP lidocaine infusions did not have any motivational effects of their own. We conclude therefore that TPP inactivation directly interferes with the reward produced by morphine. Furthermore, to investigate the possibility that reversible TPP inactivation produces a general attention or learning deficit, the aversive properties of nicotine were examined. We observed that intra-TPP lidocaine infusion did not block the acute aversion produced by nicotine. This result confirms that reversible TPP inactivation does not block attention or the general ability to learn place conditioning (Bechara & van der Kooy, 1992; Bechara *et al.*, 1992, 1995; Parker & van der Kooy, 1995; Bechara *et al.*, 1998).

In order to explore if the second opponent process is similar to the pharmacological effect of morphine leaving the brain and blood, and detaching from opiate receptors, the aversive properties of the opioid antagonist naloxone were tested. We observed that naloxone aversions were blocked by reversible TPP inactivation. However, reversible inactivation of the TPP during withdrawal did not block the conditioned place aversion induced by 16 h spontaneous withdrawal from an acute morphine injection in non-dependent rats. This result implies that the aversive effects of acute morphine withdrawal and naloxone aversion are fundamentally different. This suggests that the opponent process is not simply a mirror of the morphine or endogenous opioids being directly displaced from opiate receptors or cleared from the body. Thus, the aversive morphine withdrawal state in non-dependent rats is related to a neuronal change caused by the acute rewarding effect of morphine.

In previous studies it has been observed that TPP lesions do not block the aversion produced by naloxone (Bechara *et al.*, 1995; Vargas-Perez *et al.*, 2007), which is blocked by mediobasal arcuate hypothalamic lesions (Mucha *et al.*, 1985). This divergence between the reversible TPP inactivation and TPP excitotoxic lesions is not presently understood. However, analogous discrepancies are observed between excitotoxic lesions and lidocaine-induced reversible inactivation in other neural systems. In these studies, a series of compensatory neuronal plasticity mechanisms that take place after excitotoxic lesions are suggested to explain these discrepancies (Arvanitogiannis *et al.*, 1996; Waraczynski & Perkins, 1998; Lomber, 1999; Waraczynski *et al.*, 1999; Acheson *et al.*, 2000; Waraczynski & Perkins, 2000).

The TPP lesions disrupt the opponent process by blocking the rewarding properties of morphine in non-dependent rats (Vargas-Perez *et al.*, 2007). The present results demonstrate that the TPP is not directly mediating the opponent process itself. According to opponent-process theory, the two opposing responses are linked but they should depend on different neurobiological mechanisms because the second process has a longer latency, weaker intensity and slower decay than the first process (Solomon & Corbit, 1974; Solomon, 1980). We investigated whether the second opponent process takes place within the same brain region as the first process and indeed it did; whether it reflects a reversal in the activity of the neuronal system that mediates the first process is unclear. The present results reveal that spontaneous aversive morphine with-

drawal takes place in a completely different neuronal system than the neuronal system in the TPP, which mediates the rewarding effects of morphine. This different non-TPP neuronal system should be triggered by the primary response to the acute morphine in the TPP and should produce the adaptation(s) that results in the aversion to morphine withdrawal. It is not clear which other system (or systems) controls the aversive second process. It is clear that disruption of midbrain dopaminergic transmission has no effect on spontaneous morphine withdrawal aversions in non-dependent rats (Bechara *et al.*, 1995). However, other studies implicate the amygdala and a dopamine-independent component of the nucleus accumbens as potential substrates for the aversive stimulus effects of opiate withdrawal in non-dependent animals (Koob *et al.*, 1989b; Stinus *et al.*, 1990; Koob *et al.*, 1992). Still other studies identify the bed nucleus of the stria terminalis as being critical for the affective component of aversive stimuli such as opiate withdrawal (Aston-Jones *et al.*, 1999; Delfs *et al.*, 2000), stress (Cecchi *et al.*, 2002) and nociception (Deyama *et al.*, 2008). Our results show that the TPP does not directly mediate the morphine spontaneous withdrawal aversion and suggest that a different system, triggered by the changes in the TPP after the primary drug response, produces the aversion itself.

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Abbreviations

PBS, phosphate-buffered saline; TPP, tegmental pedunculopontine nucleus.

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