

A test of the opponent-process theory of motivation using lesions that selectively block morphine reward

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Abstract

The opponent-process theory of motivation postulates that motivational stimuli activate a rewarding process that is followed by an opposed aversive process in a homeostatic control mechanism. Thus, an acute injection of morphine in nondependent animals should evoke an acute rewarding response, followed by a later aversive response. Indeed, the tegmental pedunculo-pontine nucleus (TPP) mediates the rewarding effects of opiates in previously morphine-naïve animals, but not other unconditioned effects of opiates, or learning ability. The aversive opponent process for acute morphine reward was revealed using a place-conditioning paradigm. The conditioned place aversion induced by 16-h spontaneous morphine withdrawal from an acute morphine injection in nondependent rats was abolished by TPP lesions performed prior to drug experience. However, TPP-lesioned rats did show conditioned aversions for an environment paired with the acute administration of the opioid antagonist naloxone, which blocks endogenous opioids. The results show that blocking the rewarding effects of morphine with TPP lesions also blocked the opponent aversive effects of acute morphine withdrawal in nondependent animals. Thus, this spontaneous withdrawal aversion (the opponent process) is induced by the acute rewarding effects of morphine and not by other unconditioned effects of morphine, the pharmacological effects of morphine or endogenous opioids being displaced from opiate receptors.

Introduction

After the acute reward triggered by psychoactive drugs, animals experience a rebound aversive state (Wise, 1996; Koob & Le Moal, 1997; Robinson & Berridge, 2003). This two-sided motivational effect is predicted by the opponent-process theory of motivation (Solomon & Corbit, 1974), which states that any motivational stimulus activates two opposing processes. The first process has a fast onset and end, 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 (Fig. 1A). In this view, positive motivational stimuli activate a dose-dependent process in brain reward circuits, which in turn causes the activation of a negative affective state or opponent second-process, which is part of a homeostatic balance that brings brain states back to normal. This theory implies that blocking the first process should also prevent the second process. Consequently it could be predicted that, if specifically the rewarding effects of morphine administration were blocked, the aversive motivational effects of acute opiate withdrawal would not occur in nondependent animals.

Withdrawal is a major unpleasant side-effect of opiate administration that occurs when opiates are cleared from an animal (Koob & Le Moal, 1997). The dramatic somatic manifestations of opiate withdrawal (including diarrhea, body shakes and jumping) are the best known aspects of the withdrawal syndrome in dependent animals

(Way *et al.*, 1969; Koob *et al.*, 1992; Koob & Le Moal, 2005). In nondependent animals the effects of opiate withdrawal are less striking (Bozarth & Wise, 1984; Bechara *et al.*, 1995). However, the aversive motivational effects of withdrawal can be revealed (albeit to a lesser degree than in dependent animals) when morphine withdrawal is paired with visual and textural environmental stimuli, such as in conditioned place-aversion studies (Mucha *et al.*, 1982; van der Kooy *et al.*, 1982; Bechara *et al.*, 1995; Azar *et al.*, 2003).

Although the motivational effects of withdrawal are seen in both opiate-dependent and -nondependent rats, they are fundamentally different. The acute withdrawal aversions in nondependent animals can only be detected at 11–16 h post-morphine, whereas in dependent animals the morphine withdrawal aversions last longer (11–24 h) and are more prominent (Bechara *et al.*, 1995). Furthermore, there is evidence to suggest that the conditioned place aversions produced by morphine withdrawal in the drug-dependent vs. -nondependent states depend on different brain mechanisms (Bozarth & Wise, 1984; Bechara *et al.*, 1995). The aversion produced by morphine withdrawal in opiate-dependent animals is mediated by a dopaminergic pathway (Di Chiara & Imperato, 1988; Koob *et al.*, 1992; Nestler, 1993; Bechara *et al.*, 1995; Wise, 1996; Dockstader *et al.*, 2001; Laviolette *et al.*, 2002). In particular, disrupting dopaminergic signalling with either neuroleptics or mutations on dopamine D2 receptors blocks the conditioned place aversions produced by morphine withdrawal in opiate-dependent animals (Bechara *et al.*, 1995; Dockstader *et al.*, 2001). However, the same manipulations do not block the aversive effects of acute morphine withdrawal in nondependent animals (Bechara *et al.*, 1995; Dockstader *et al.*, 2001). The tegmental

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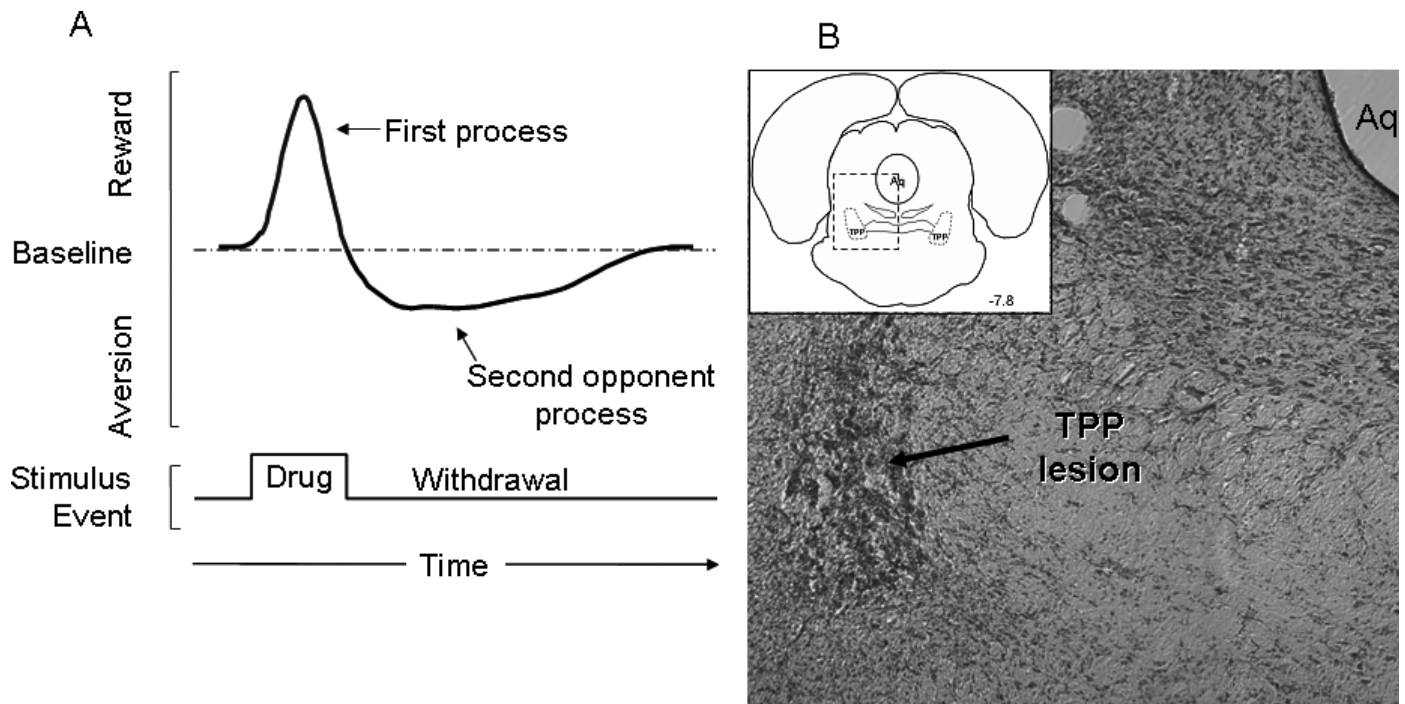


FIG. 1. The opponent-process model for morphine motivation in nondependent rats and photomicrograph of a representative TPP lesion. (A) Drug intake arouses a positive first process (reward) that in turn elicits a negative second 'opponent process' (aversion). (B) Coronal section (10 \times magnification) of a cresyl violet-stained section in the region of the TPP. Inset, schematic of the approximate anatomical region from which the section displayed was taken; Aq, aqueduct.

pedunculopontine nucleus (TPP) of the brainstem is a critical neural substrate underlying the motivational effects of opiates in nondependent animals (Bechara & van der Kooy, 1992; Olmstead & Franklin, 1993). However, the TPP is not involved in the regulation of other effects of acute morphine administration in nondependent animals, such as conditioned taste aversions (Bechara *et al.*, 1993) or analgesia (Bechara & van der Kooy, 1992). According to the opponent-process theory, TPP lesions should block the aversive response shown by nondependent animals to spontaneous opiate withdrawal due to their ability to block the acute rewarding effects of morphine. Conversely, if withdrawal aversions are the result of other unconditioned effects of morphine, such as the pharmacological effect of morphine being displaced from its opiate receptor and leaving the brain and blood, then TPP lesions should not block withdrawal aversions.

In the present study, a possible role of the TPP in the aversive effects of spontaneous withdrawal from acute morphine administration was assessed in nondependent rats. Using an unbiased place-conditioning paradigm, we examined the effects of bilateral lesions of the TPP on the motivational properties of spontaneous withdrawal. As in the case of opiate reward, TPP lesions blocked the conditioned place aversion to morphine withdrawal in nondependent animals but, in concordance with previous studies (Bechara *et al.*, 1995), did not block the general ability to learn conditioned place aversions.

Materials and methods

Animals

Subjects were male Wistar rats (Charles River) weighing 350–500 g during experimental training. Rats 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. All experimental protocols were approved by and

conformed to the Institutional and Governmental Animal Care Committee guidelines and coincided with principles of animal care (Faculty Advisory committee on animal services).

Excitotoxic TPP lesions

Rats were anaesthetized with sodium pentobarbital (30 mg/kg *i.p.*) (Somnotol; MTC pharmaceuticals, Canada) and placed in a stereotaxic device. Bilateral TPP lesions ($n = 12$) were induced by microinfusing NMDA (0.1 M; Sigma–Aldrich, St Louis, MO) in a volume of 0.20 μ L of physiological saline. Sham-lesioned control rats ($n = 8$) received bilateral injections of the physiological saline vehicle. The injection coordinates for the TPP (taken from Paxinos & Watson, 1986) were, bilaterally, 7.8 mm posterior to bregma, 1.6 mm lateral to the midline and 6.6 mm below the dura. Microinfusions were performed with a 1- μ L Hamilton (Reno, NV, USA) microsyringe for a 20-min period per hemisphere. The infusion rate for NMDA was 0.01 μ L/min, after which the injector was left in place for an additional 5 min to allow diffusion of the solution from the injector tip (Kippin & van der Kooy, 2003). At least 2 weeks were allowed for postsurgical recovery prior to behavioural training.

Histological analysis

At the end of experiments, rats were deeply anaesthetized 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 40- μ m-thick sections, and mounted on gelatine-coated slides. Sections of the TPP were processed for cresyl violet staining and subsequently examined by light microscopy.

Place-conditioning apparatus

The place-conditioning apparatus was identical to that described previously (Mucha *et al.*, 1982; Bechara & van der Kooy, 1992), as was the conditioning procedure (Bechara & van der Kooy, 1992; Bechara *et al.*, 1992). Briefly, conditioning took place in one of two (41 × 41 × 38 cm) distinct boxes, which differed in colour, texture and smell. During testing, each rat was placed in a narrow neutral grey zone (41 × 10 cm) that separated the two compartments and was allowed to explore both environments freely for a period of 10 min. This place-conditioning apparatus has been shown to yield equal preferences for the two environments (Mucha *et al.*, 1982; Mucha & Iversen, 1984). When uninjected or vehicle-injected nondependent rats were repeatedly placed in only one environment of this place-conditioning apparatus, these rats showed equal preferences for the familiar environment and the unfamiliar novel environment when later allowed to explore both environments freely for 10 min (Mucha *et al.*, 1982; Mucha & Iversen, 1984; Bechara & van der Kooy, 1992; Bechara *et al.*, 1992).

Conditioning and training

To assess the effect of TPP lesions on the aversive motivational effect of spontaneous withdrawal from morphine, we have employed a modified place-conditioning procedure for which conditioning took place in only one compartment (Procedure W or withdrawal-paired) of the place-conditioning apparatus (Bechara *et al.*, 1992, 1995). This single-side-withdrawal procedure of place conditioning 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). Briefly, each nondependent rat was given morphine (20 mg/kg i.p.) and returned immediately to its home cage. Approximately 16 h later, the 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. Testing, as previously described, took place 2 days after the end of conditioning; all rats were tested drug-free. One of the two compartments was previously paired with the absence of morphine and the other was an unfamiliar neutral environment. The time spent in each environment was recorded over a 10-min test period. Times spent in each environment were scored separately for each animal.

To behaviourally verify the TPP lesions, we then tested the same group of rats using the standard place-conditioning procedure. Both groups of rats were administered intraperitoneal injections of morphine (20 mg/kg). The rats received four drug–environment and four saline–environment conditioning sessions for 40 min over eight consecutive days. Exposure to environments and order of saline vs. morphine injections was fully counterbalanced. The pairing of each rat with an environment was counterbalanced with the previous experiment; thus, half of each group received morphine in the previously neutral environment and half in the previously withdrawal-paired environment. Two days later, rats were tested drug-free and the time spent in each environment was recorded over a 10-min test period.

It has been shown that TPP lesions do not block the aversive effects of naloxone in nondependent rats (Bechara *et al.*, 1995). To test for possible learning impairments in our TPP-lesioned rats, we next tested the same groups of sham and TPP-lesioned rats in the conditioned place preference paradigm with naloxone (5.0 mg/kg, i.p.). Both groups of rats were conditioned for 40 min each with four drug–environment and four saline–environment sessions spread equally over 8 days. Exposure to these environments was fully counterbalanced. The pairing with the environment was also counterbalanced

with the previous experiment. Thus, half of each group that got morphine in the white environment now got naloxone in the black environment, and vice versa. Two days later, rats were tested drug-free and the time spent in each environment was recorded over a 10-min test period.

After each experiment was completed, an extinction conditioning procedure was performed (Laviolette & van der Kooy, 2004). Briefly, at least one month after each experiment was completed, rats were placed in a drug-free state into the conditioning boxes, in a fully counterbalanced order. On day 1, the rats were placed into one of the conditioning environments for 2 h then returned to their home cages for 1 h. After that, the rats were placed into the alternate conditioning environment for 2 h, and then returned to their home cages. This cycle was repeated over 2 days. Thus, rats were exposed to each environment for a total of 4 h over the 2-day extinction period. On the third day, the rats were given an extinction test and were given free access to both environments for a 10-min period. Time spent in each of the environments was scored separately for each animal. Rats that displayed no preference for either of the environments were considered extinguished.

The place conditioning produced by spontaneous withdrawal aversion was completely extinguished and rats displayed no aversion prior to retraining (mean ± SEM in sham animals: time spent in previous unconditioned environment, 242.68 ± 39.17 vs. previous conditioned environment, 210.36 ± 35.52 s ($t_7 = 0.49$, $P > 0.05$). Mean ± SEM in TPP-lesioned animals: previous unconditioned, 227.13 ± 36.29 vs. previous conditioned environment, 251 ± 42.37 s ($t_8 = 0.44$, $P > 0.05$). In the same way, place conditioning produced by morphine was completely extinguished and rats displayed no preference prior to retraining (mean ± SEM in sham animals: previous morphine-paired environment, 221.9 ± 68.52 vs. previous saline-paired environment, 298.46 ± 68.90 s ($t_7 = 0.75$, $P > 0.05$). Mean ± SEM in TPP-lesioned animals: previous morphine-paired side, 208.89 ± 51.25 vs. previous saline paired side, 267.62 ± 55.09 s ($t_8 = 0.75$, $P > 0.05$).

To test whether TPP lesions altered general reward value perception (i.e. anhedonia), rats were tested for sucrose preference (Shimura *et al.*, 2002; Martinez-Hernandez *et al.*, 2006). Non-water-deprived rats were given simultaneous access to one bottle containing water and another containing 0.1 M sucrose in water. The location (left or right side) of the bottles containing each liquid was randomly assigned for each animal. We measured the intake of water and sucrose solution during a 48-h period by weighing both bottles before and after the test (Martinez-Hernandez *et al.*, 2006).

Statistical analyses

All data were analysed by two-tailed paired Student's *t*-tests. Due to the multiple comparisons made on each experiment, the Bonferroni correction was performed.

Results

Histological analysis

All TPP-lesioned rats included in statistical analyses of the behavioural data were verified histologically to confirm that the lesion destroyed the majority of the neurons in the TPP nucleus bilaterally. Three rats were excluded following histological analysis because of either incomplete lesions or lesion damage extending beyond the boundaries of the TPP. Figure 1B shows a photomicrograph of a representative TPP lesion.

The aversive effects of acute morphine withdrawal were blocked by TPP lesions

In sham-lesioned rats ($n = 8$), 16 h abstinence from morphine (20 mg/kg) produced a robust conditioned place aversion for the spontaneous withdrawal-paired environment. In contrast, TPP-lesioned rats ($n = 9$) did not exhibit conditioned place aversion for the withdrawal-paired compartment (Fig. 2A). A significant difference between the times spent during testing on the unfamiliar neutral vs. withdrawal-paired (saline vehicle) compartments was seen in the sham rats ($t_7 = 7.056$, $P < 0.05$). Conversely, there was no significant difference between the times spent during testing on the unfamiliar neutral vs. withdrawal-paired compartments in TPP-lesioned rats ($t_8 = 0.999$, $P > 0.05$). Thus sham, but not TPP-lesioned, rats showed significant aversions for places paired with acute morphine withdrawal.

TPP lesions blocked acute morphine reward

Morphine (20 mg/kg, i.p.) produced conditioned place preferences in rats with sham but not with excitotoxic lesions of the TPP (Fig. 2B). There was a significant difference between the times spent in the previously morphine- vs. saline vehicle-paired compartments in sham rats ($t_7 = 5.034$, $P < 0.05$), but not in TPP-lesioned rats ($t_8 = 0.768$, $P > 0.05$).

TPP lesions did not block acute naloxone aversion

The acute aversive properties of naloxone in place conditioning were not blocked by TPP lesions (Fig. 2C). There was a significant difference between the times spent during testing in the previously

naloxone- vs. saline vehicle-paired compartments in sham rats ($t_7 = 7.317$, $P < 0.05$) and in TPP-lesioned rats ($t_8 = 5.837$, $P < 0.05$).

TPP lesions did not block sucrose consumption

The preference for sucrose consumption was not blocked by TPP lesions (Fig. 2D). A significant difference between tap water and sucrose consumption was seen in both sham rats ($t_7 = 4.448$, $P < 0.05$) and in TPP-lesioned rats ($t_8 = 12.737$, $P < 0.05$).

Discussion

Even the first administration of morphine produces a biphasic pattern of motivational changes in animals. The opponent-process theory of motivation posits that the onset of the drug produces a reward 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 (Solomon & Corbit, 1974). We tested the opponent-process theory using lesions known to disrupt the rewarding properties of acute opioid administration in nondependent rats. Indeed, TPP lesions blocked both the acute rewarding and the aversive withdrawal effects produced by morphine administration in nondependent rats. In a behavioural verification of the TPP lesions, acute morphine conditioned-place preferences were blocked by the TPP lesions in the same rats. The data support the conclusion that the same neuronal substrate (the TPP) that mediates acute opioid reward in nondependent rats plays a key role in spontaneous withdrawal aversion. In other words, when the rats were unable to sense the reward state related to acute morphine administration, the aversive second opponent process did

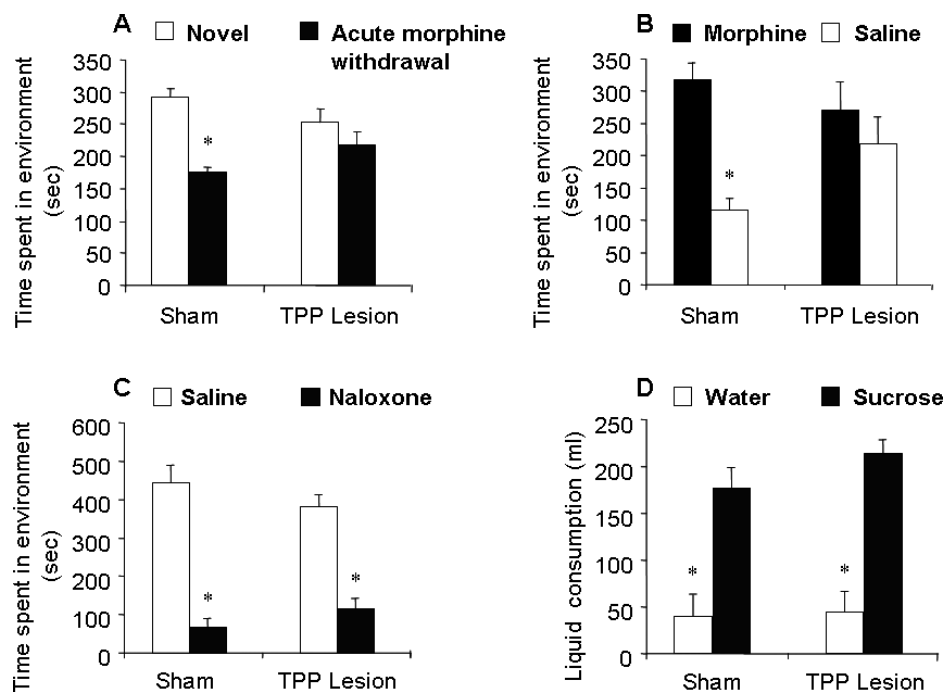


FIG. 2. Effects of bilateral sham ($n = 8$) or excitotoxic lesions ($n = 9$) of the TPP on motivated behaviours in nondependent rats. (A) TPP lesions blocked the aversions for places paired with acute morphine withdrawal. Data represent the mean \pm SEM of the absolute time spent on the previously unfamiliar novel and morphine withdrawal-paired compartments ($*P < 0.05$). (B) Morphine-conditioned place preferences were blocked by TPP lesions. Data represent mean \pm SEM of the absolute time spent in the previously morphine- and previously saline-paired compartments during testing ($*P < 0.05$). (C) TPP lesions did not block conditioned place aversions induced by naloxone. Data represent the mean \pm SEM of the absolute time spent in the previously saline- and previously naloxone-paired compartments ($*P < 0.05$). (D) TPP lesions did not block the preference for sucrose vs. water consumption over 48 h ($*P < 0.05$).

not emerge. This suggests that the aversive state related to acute opiate withdrawal depends on the previous rewarding state evoked by acute morphine administration.

It is possible that TPP-lesioned rats have an altered perception of general reward value, producing an acute decrease in sensitivity to reward (Zacharko & Anisman, 1991). As a consequence, a disruption of the aversive second opponent process could be due to the blockade of the general mechanism of reward in the TPP-lesioned rats. However, TPP lesions did not interfere with a preference for sweet tastes, showing that TPP lesions do not interfere with rats' general perception of reward (Keating *et al.*, 2002). Furthermore, it has been shown that TPP lesions do not block opiate reward in drug-dependent rats (Bechara & van der Kooy, 1992; Bechara *et al.*, 1992, 1998), nor do they block the rewarding effects of other nonopiate drugs such as cocaine (Parker & van der Kooy, 1995). Contrary to producing a general anhedonic state, TPP lesions interfere with the reward mediated by morphine only in nondependent rats. Thus, we conclude that the blockade of the opiate withdrawal aversion in these rats is probably the result of blocking acute opiate reward.

TPP lesions block spontaneous morphine withdrawal in nondependent rats but the same lesions do not block an aversion to naloxone. These results confirm that TPP lesions do not cause general attentional or learning deficits (Bechara & van der Kooy, 1992; Bechara *et al.*, 1992, 1995, 1998; Parker & van der Kooy, 1995). These experiments also reveal that the aversions produced by the acute termination of endogenous opioids mediated by naloxone are not blocked by TPP lesions. Indeed, these acute naloxone aversions are blocked by lesions of the mediobasal arcuate hypothalamus (Mucha *et al.*, 1985). This suggests that the aversive morphine withdrawal state in nondependent rats is not due to the pharmacological effect of endogenous opioids directly being displaced from opiate receptors by naloxone. Indeed, the aversion to acute morphine withdrawal in nondependent rats only can be observed 11–16 h after the last injection of morphine, many hours after morphine has already been cleared from the brain and blood (Bechara *et al.*, 1995). Thus, this aversion is probably related to the neurobiological changes (the opponent process) caused by the reward during morphine administration, and is not related to the neurobiological effects caused by endogenous opioids and presumably morphine leaving the brain and blood. We suggest that the opponent process is produced by morphine acting on opiate receptors and not by either morphine or endogenous opioids being displaced from their binding to opiate receptors.

In nondependent rats, the aversive morphine-induced opponent process appears to be mediated by the TPP and is not dependent on the acute aversive effects of morphine as measured by the conditioned taste aversion paradigm (Bechara *et al.*, 1993). The acute aversive effects produced by morphine in the conditioned taste aversion paradigm are mediated by dopamine neurons and not the TPP (Zito *et al.*, 1988; Bechara *et al.*, 1993). TPP lesions, however, are sufficient to disrupt the opponent process related to morphine motivation in nondependent rats. In contrast, in drug-dependent rats the aversive opponent process appears to involve more neuronal systems than those that are directly affected by the rewarding effects of morphine, or the neurobiological substrate(s) mediating this process are more diffusely distributed (Koob & Bloom, 1988; Koob *et al.*, 1992; Wise, 1996; Robinson & Berridge, 2003). Indeed, dopamine neurons, and not the TPP, are involved in the aversive effects of withdrawal in opiate-dependent rats (Bechara *et al.*, 1995). These data suggest that the two different opponent processes mediating withdrawal aversions in nondependent vs. opiate-dependent rats are fundamentally different.

The TPP nucleus is directly involved in mediating the opponent process triggered by morphine administration in nondependent rats.

However, the cellular and neurobiological mechanisms that underlie the activation of the aversive second process after morphine reward in these rats have not been elucidated. One possibility is that the neurons in the TPP adapt in order to neutralize the acute effects of the morphine. Consequently, any of the main cellular groups of the TPP, including the cholinergic cell group Ch5, glutamatergic, GABAergic and peptidergic cell types (Mena-Segovia *et al.*, 2004), and/or the interaction among them, could be being affected during the acute morphine administration, and then adapt to produce the opponent process. Thus, the persistence of the adaptation in the TPP after the morphine clears the organism would be the cause of the aversive withdrawal response. This could be tested by reversibly inactivating the TPP 16 h after acute morphine administration to see whether this manipulation blocks the acute morphine withdrawal aversions. An alternate explanation is that a different system, triggered by the changes in the TPP after the primary drug response, produces the aversion after the acute morphine reward. Although it is not clear which other system (or systems) might be involved in the withdrawal effect of morphine in nondependent animals, the dopaminergic system is unlikely to be involved as disruption of midbrain dopaminergic transmission has no effect on spontaneous morphine withdrawal in the nondependent condition (Bechara *et al.*, 1995).

In summary, the data presented here show that in nondependent rats the TPP serves as a nodal brainstem substrate for the mechanism underlying the motivational effects of both acute morphine reward and spontaneous withdrawal aversion, its opponent process. As such, these findings support the opponent-process theory.

Abbreviation

TPP, tegmental pedunculopontine nucleus.

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