

# GABA<sub>A</sub> receptors signal bidirectional reward transmission from the ventral tegmental area to the tegmental pedunculo-pontine nucleus as a function of opiate state

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## Abstract

The brainstem tegmental pedunculo-pontine nucleus (TPP) is involved in reward signalling and is functionally and anatomically linked to the VTA. We examined the possible role of the TPP as a reward transmission output for GABA<sub>A</sub> receptors in the VTA in rats not previously exposed to opiates vs. rats that were chronically exposed to and in withdrawal from opiates or in rats that had recovered from chronic opiate exposure. Bilateral lesions of the TPP blocked the rewarding effects of a GABA<sub>A</sub> antagonist but not the rewarding effects of a GABA<sub>A</sub> receptor agonist in rats previously unexposed to opiates. This functional pattern was reversed in rats that were dependent on opiates and in withdrawal. However, once rats had recovered from chronic opiate exposure the functional parameters of VTA GABA<sub>A</sub> receptor reward signalling reverted to the pattern observed in animals that had not been exposed to opiates. These findings suggest that GABA<sub>A</sub> receptors in the VTA can regulate differential reward signalling through separate neural systems during the transition from a drug-naïve to a drug-dependent and withdrawn state.

## Introduction

The ventral tegmental area (VTA) is involved importantly in neural reward signalling (Laviolette & van der Kooy, 2001; Steffensen *et al.*, 1998). Within the VTA GABA<sub>A</sub> receptors serve to transmit reward information and this has been demonstrated both in the intracranial drug self-administration (ICSA) paradigm (David *et al.*, 1997; Ikemoto *et al.*, 1997, 1998) and place conditioning procedure (Laviolette & van der Kooy, 2001, 2004). The VTA also sends limited descending inputs to the brainstem tegmental pedunculo-pontine nucleus (TPP) region (Semba & Fibiger, 1992; Steininger *et al.*, 1992) although these projections do not appear robust. The TPP is also involved importantly in reward signalling. TPP lesions block the rewarding effects of opiates, nicotine, food and sex (Bechara & van der Kooy, 1992; Olmstead & Franklin, 1994; Olmstead *et al.*, 1999; Laviolette *et al.*, 2002; Kippin & van der Kooy, 2003). Interestingly, TPP lesions block acute opiate reward in drug-naïve animals (Nader & van der Kooy, 1997; Olmstead & Franklin, 1994; Olmstead *et al.*, 1999), but not in animals that are in a drug-dependent and withdrawn state (Nader & van der Kooy, 1997; Olmstead *et al.*, 2001).

Within the VTA, GABA<sub>A</sub> receptors associated with GABAergic neurons provide inhibition to DAergic neurons and send efferents from the VTA (Semba & Fibiger, 1992; Sesack & Pickel, 1995; Van Bockstaele & Pickel, 1995). These receptors can gate bidirectional transmission of reward signals through either a neural reward system that does not require DA signalling or a separate, mesolimbic DAergic

neural motivational system (Laviolette & van der Kooy, 2001, 2004). However, the neural system that mediates VTA reward through a non-DA mechanism is not presently understood.

Given that TPP lesions block intra-VTA opiate reward (Nader & van der Kooy, 1997), this may suggest a reward-signalling link between these regions via a descending pathway from the VTA to the TPP. To examine the possible role of VTA GABA<sub>A</sub>-receptor reward signalling through the brainstem TPP and how this pathway may be affected by opiate state, we examined the rewarding effects of a GABA<sub>A</sub> agonist or antagonist in the VTA following bilateral excitotoxic lesions of the TPP in either opiate-naïve, opiate-dependent and withdrawn, or animals that had recovered from chronic opiate exposure.

## Materials and methods

### *Animals and surgery*

Male Wistar rats (Charles River) weighing 300–350 g at the start of the experiments were anaesthetized with sodium pentobarbital (Somnotol, 0.8 mL/kg, i.p.) and placed in a stereotaxic device with the incisor bar set to –3.3 mm. Twenty-two-gauge stainless steel guide cannulae (Plastics One, Roanoke, VA, USA) were implanted bilaterally 2 mm dorsal to the VTA at a 10° angle using the following stereotaxic coordinates from bregma (in mm): AP, –5.0; L, ±2.3; and, from the dural surface, V, –8.0. All coordinates were taken from the atlas of Paxinos & Watson (1986). Cannulae were secured to the skull surface with jeweler's screws and dental acrylic. All experimental protocols conformed to Institutional and Governmental Animal Care guidelines.

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### Excitotoxic TPP lesions

Lesions of the TPP ( $n = 31$ ) were performed bilaterally by injecting NMDA (0.1 M; Sigma) in a volume of 0.20  $\mu$ L of physiological saline (pH adjusted to 7.4). Sham-lesioned control rats ( $n = 33$ ) received bilateral injections of the same volume of physiological saline vehicle. NMDA microinfusions were performed with a 1- $\mu$ L Hamilton (Reno, NV, USA) microsyringe for a 20-min period per hemisphere. The infusion rate was 0.02  $\mu$ 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. The injection coordinates for the TPP were from bregma (in mm): AP,  $-7.8$ ; L,  $\pm 1.6$ ; and from the dural surface, V,  $-6.6$ . For rats receiving both intra-VTA cannulae and TPP lesions, the surgical procedures were performed simultaneously. Rats were given at least 2 weeks of recovery time before any conditioning procedures were begun.

### Experimental groups and procedures

#### First experiment

In the first experiment, eight groups of rats that were not lesioned received, intra-VTA, either muscimol or bicuculline. Four of the groups were conditioned with intra-VTA muscimol (5 or 50 ng) in either an opiate-naive state ( $n = 8$ ,  $n = 8$ , respectively) or opiate-dependent and withdrawn state ( $n = 8$ ,  $n = 7$ , respectively). The other four groups received intra-VTA bicuculline (5 or 50 ng) in either the opiate-naive ( $n = 8$ ,  $n = 9$ , respectively) or opiate-dependent and withdrawn state ( $n = 9$ ,  $n = 8$ , respectively). For all the experiments in opiate-dependent and withdrawn rats, opiate dependence and withdrawal was induced by treating rats for 4 days prior to conditioning with a high dose (0.5 mg/kg) of heroin (s.c.) Rats conditioned in an opiate-dependent and withdrawn state received intra-VTA microinfusions 21 h after the last heroin injection. Thus, during the training phase, 21 h elapsed between the heroin injection and the training session. Subsequent maintenance doses of heroin were administered 3.5 h after the conditioning session. By the completion of training, heroin-treated animals had received a total of 21 heroin injections. After this, the rat was left in the home cage for 7 days before being tested for CPP. This heroin treatment regimen induced opiate dependence indicated by the presence of somatic and aversive opiate withdrawal and strong motor sensitization by the third heroin exposure. The aversive effects of opiate withdrawal induced by this regimen was equivalent to that observed following a 3-week chronic morphine exposure regimen (Bechara *et al.*, 1992). For the groups that were conditioned in the opiate-naive state, saline injections were performed in place of the heroin injections according to the same schedule outlined above.

#### Second experiment

In the second experiment we examined the effects of intra-VTA bicuculline (50 ng) or muscimol (50 ng) on the aversive effects of spontaneous heroin withdrawal in separate groups of nonlesioned rats that were in the opiate-dependent and withdrawn state (see above). We used a single-sided withdrawal place-conditioning procedure. The single-sided withdrawal procedure (Bechara & van der Kooy, 1992) involves exposing the rat to only one of the two conditioning environments. In this procedure, rats are given a heroin injection and returned to the home cage. Twenty-four hours after the last drug injection, rats are placed in one of the two conditioning environments for 40 min. The environment paired with the absence of heroin is counterbalanced within groups. Previous studies have shown that,

21 h postinjection, rats that have been chronically treated with opiates experience the aversive motivational effects of withdrawal and display aversions to environments paired with opiate withdrawal (Bechara *et al.*, 1992). This conditioning cycle is repeated until each rat has been exposed to the conditioning environment four times over 8 days. Three groups of rats were conditioned in this way and received, intra-VTA, either saline ( $n = 8$ ), muscimol ( $n = 7$ ) or bicuculline ( $n = 7$ ).

#### Third experiment

In the third experiment, four groups of animals received bilateral TPP lesions and four groups received saline sham lesions of the TPP. Two of the lesioned groups were assigned to the intra-VTA muscimol (50 ng) conditions and were either trained while opiate-naive ( $n = 7$ ; sham controls,  $n = 8$ ) or opiate-dependent and withdrawn ( $n = 7$ ; sham controls,  $n = 8$ ). Two more lesioned groups were assigned to the intra-VTA bicuculline condition and were trained while opiate-naive ( $n = 9$ ; sham controls,  $n = 8$ ) or opiate-dependent and withdrawn ( $n = 8$ ; sham controls,  $n = 8$ ).

#### Fourth experiment

In the fourth experiment, rats that had previously been trained in the opiate-dependent withdrawn state had their training extinguished and were retrained after being randomly reassigned to either the bicuculline or muscimol conditions. For the groups that were recovered from opiate exposure, extinguished and retrained in the opiate non-dependent state, an extinction conditioning procedure was used that involved placing the animals (in a drug-free state) into the conditioning boxes, in a fully counterbalanced order. On day 1, the animals were placed into one of the conditioning environments for 2 h then returned to their home cages for 1 h. Following this, the animals were placed in the alternate conditioning environment for 2 h then returned to the home cages. This cycle was repeated over 2 days. Thus rats were exposed to each environment (in a drug-free state) for a total of 4 h over the 2-day extinction training. On the third day, animals were given an extinction test and were given free access to both environments for a 10-min period, as described previously. Time spent in each of the environments was scored separately for each animal. Animals that displayed no preference for either of the environments were considered extinguished and, 2 days later, retraining of the animals with, intra-VTA, either bicuculline (50 ng;  $n = 8$ ) or muscimol (50 ng;  $n = 7$ ) in the opiate-recovered state (animals were randomly assigned to either drug group) was begun, and proceeded exactly as described previously. Thus 10 days passed between the last heroin exposure and subsequent retraining postextinction.

#### Histological analysis

At the end of experiments, rats were deeply anaesthetized with sodium pentobarbital and perfused transcardially with 200 mL of phosphate-buffered saline (0.1 M) followed by 400 mL of 4% paraformaldehyde. Brains were rapidly removed and stored for 16 h in a 4% paraformaldehyde postfixative. After postfixation, brains were transferred to a 25% sucrose solution in phosphate buffer (0.1 M) for 24 h. Brains were then flash-frozen at  $-70$  °C, sliced in a freezing microtome in 40- $\mu$ m-thick sections and mounted on gelatin-coated slides. Alternate sections of the TPP were processed separately for either Cresyl Violet staining or for NADPH histochemical staining (described below). Intra-VTA cannulae placements were verified with light microscopy using the atlas of Paxinos & Watson (1986). NADPH histochemical analysis of TPP lesions was performed according to the method of

Vincent *et al.* (1983). Mounted sections of the TPP were preincubated for 10 min in 0.003% Triton X-100 in phosphate buffer (0.1 M). Sections were then rinsed in phosphate buffer and incubated for 3.5–5 h in the NADPH-diaphorase staining solution, which consisted of 0.5 mg/mL  $\beta$ -NADPH (Sigma) and 0.2 mg/mL of nitroblue tetrazolium (Sigma) in 0.003% Triton X-100 in phosphate buffer (0.1 M). Slides were then rinsed in 0.3 M phosphate buffer for three rinses, allowed to dry and then rinsed in graded alcohols. Slides were coverslipped with Permount. This histochemical technique selectively stains for the cholinergic cells of the reticular brainstem (Vincent *et al.*, 1983). Lesions of the TPP or bilateral cannulae placements in the VTA that were outside the anatomical boundaries of the TPP or VTA as defined by Paxinos & Watson (1986) were excluded from analysis.

### Place conditioning

To examine the motivational effects of intra-VTA muscimol or bicuculline, rats were conditioned with an unbiased standard place-conditioning procedure (Nader & van der Kooy, 1997). Conditioning took place in one of two distinct environments that differed in colour, texture and smell. One environment was white with a wire mesh floor that was covered in wood chips. The other environment was black with a smooth Plexiglas floor that was wiped down with a 2% acetic acid solution before each conditioning session. The dimensions of the conditioning environments were 38 × 38 × 38 cm. Rats display no baseline preference for either of these two environments (Laviolette *et al.*, 2002). Rats received four drug-environment alternating with four saline-environment conditioning sessions, and exposure to conditioning environments (as well as whether the first trial was a drug or saline trial) was fully counterbalanced across all experiments. Each conditioning session lasted 40 min. At testing, 1 week after the completion of conditioning (all animals were tested drug-free), animals were placed in a narrow, neutral grey zone that separated the two test compartments. For all experiments, times spent in each environment were scored separately for each rat for a period of 10 min.

### Drugs and microinjection procedure

For all experiments, muscimol hydrobromide (Sigma), bicuculline methiodide (Sigma) or heroin were dissolved in physiological saline (pH adjusted to 7.4). Muscimol or bicuculline were microinfused bilaterally into the VTA in a volume of 0.5  $\mu$ L. Infusions were performed slowly over 1 min and injectors were left in place for a further 1 min to ensure adequate drug diffusion from the tips.

### Data analysis

All data were analysed with one- or two-way ANOVA or Student's *t*-tests, where appropriate. *Post hoc* analyses were performed with Newman–Keuls or Fisher's least significant difference tests.

## Results

### Histological analyses: TPP lesions and intra-VTA cannulae

Histological analysis of bilateral excitotoxic lesions of the TPP revealed significant lesion-induced neuronal damage extending from the rostral to caudal extents of the anatomical boundaries of the TPP. A schematic representation of bilateral TPP lesions showing the centres

of NMDA-induced damage is presented in Fig. 1A–D for animals receiving intra-VTA bicuculline (50 ng) in either the opiate-naive (Fig. 1A) or opiate-dependent and withdrawn states (Fig. 1B) or intra-VTA muscimol (50 ng) in either the opiate-naive (Fig. 1C) or opiate-dependent and withdrawn (Fig. 1D) state. Cresyl Violet analysis revealed substantial gliotic damage that was largely restricted to the anatomical boundaries of the TPP (Fig. 2A). Histochemical analysis of NADPH-positive neurons within the TPP revealed substantial loss of NADPH-positive neurons within the TPP in lesioned (Fig. 2B and D) relative to sham-lesioned (Fig. 2C and E) rats, and quantitative analysis of NADPH-positive TPP neurons revealed significant reductions in NADPH-positive neurons in both the rostral ( $t_{12} = 4.89$ ;  $P < 0.05$ ) and caudal ( $t_{14} = 4.92$ ;  $P < 0.05$ ) TPP regions relative to sham-lesioned control rats (Fig. 2F). Histological analysis of VTA sections revealed bilateral placements of VTA microinjectors within the rostral and caudal regions of the VTA. A representative sample of bilateral VTA placements are presented in Fig. 1E. Any VTA cannulae placements found outside of the anatomical boundaries of the VTA as defined by Paxinos & Watson (1986) were excluded from analysis. The VTA coordinates (see Materials and methods) used in the present study produce anatomically specific behavioural effects in the VTA (Nader & van der Kooy, 1997; Laviolette & van der Kooy, 2001, 2003). In addition, injector placements immediately dorsal or caudal to these VTA locations are behaviourally ineffective (Laviolette & van der Kooy, 2001, 2003).

### Pharmacological activation or blockade of GABA<sub>A</sub> receptors in the ventral tegmental area produced reward in the opiate-naive or opiate-dependent and withdrawn states

In separate groups of nonlesioned rats, we examined the motivational effects of intra-VTA infusions of the GABA<sub>A</sub> receptor agonist muscimol (5–50 ng) or antagonist bicuculline (5–50 ng) in either opiate-naive, or opiate-dependent and withdrawn rats. Both intra-VTA muscimol and intra-VTA bicuculline produced robust conditioned place preferences (CPP) over an order-of-magnitude dose range in both opiate-naive and opiate-dependent and withdrawn groups of rats (Fig. 3A and B). For intra-VTA muscimol, three-way ANOVA revealed a significant effect of treatment (intra-VTA: saline,  $n = 8$  vs. muscimol,  $n = 8$ ) on times spent in environments previously paired with either muscimol or saline ( $F_{1,56} = 125.6$ ;  $P < 0.05$ ), but no effect of motivational state (opiate-naive vs. dependent and withdrawn:  $F_{1,56} = 0.16$ ,  $P > 0.05$ ) or dose (5 vs. 50 ng:  $F_{1,56} = 0.09$ ,  $P > 0.05$ ) on place conditioning scores. *Post hoc* analysis revealed that rats spent significantly greater time in the muscimol-paired environment than the saline-paired environment at all doses tested (all  $P < 0.05$ ; Fig. 3A). For intra-VTA bicuculline, three-way ANOVA revealed a significant effect of treatment (intra-VTA: saline,  $n = 8$  or bicuculline,  $n = 9$ ) on times spent in environments previously paired with either bicuculline or saline ( $F_{1,54} = 121.4$ ;  $P < 0.0001$ ), but no effect of motivational state (opiate-naive vs. dependent and withdrawn:  $F_{1,54} = 0.10$ ,  $P > 0.05$ ) or dose (5 vs. 50 ng:  $F_{1,54} = 0.06$ ,  $P > 0.05$ ) on place conditioning scores. *Post hoc* analysis revealed that rats spent significantly greater time in the bicuculline-paired environment than the saline-paired environment at all doses tested (all  $P < 0.05$ ; Fig. 3B). Thus, both a GABA<sub>A</sub> agonist or antagonist produce robust rewarding effects in the VTA. In addition, both muscimol and bicuculline produce rewarding effects of similar magnitude over an order-of-magnitude dose range regardless of opiate state (Fig. 3A and B). Importantly, doses <5 ng of either bicuculline or muscimol produce no significant behavioural effects in the VTA, while doses

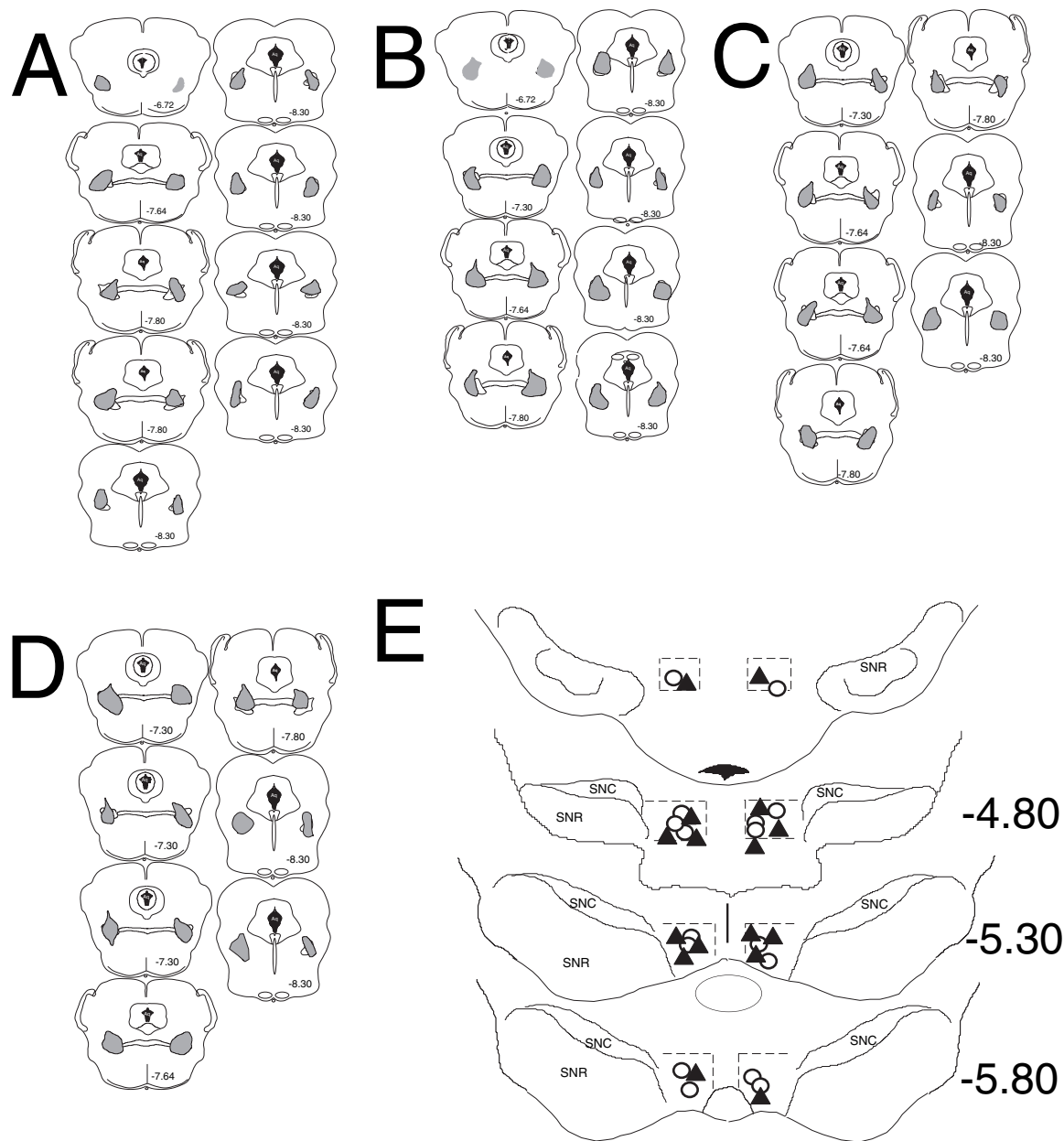


FIG. 1. Histological analyses of bilateral TPP lesions and VTA cannulae placements. A schematic representation of bilateral TPP lesions showing the centres of maximal NMDA-induced TPP damage for each individual rat. (A and B) Rats receiving intra-VTA bicuculline (50 ng) in (A) the opiate-naïve or (B) opiate-dependent and withdrawn states. (C and D) Rats receiving intra-VTA muscimol (50 ng) in (C) the opiate-naïve or (D) opiate-dependent and withdrawn state. (E) Representative schematic showing locations of bilateral intra-VTA cannulae tip locations. Microinjector tips were found in rostral and caudal regions of VTA and no observable behavioural differences were observed across these sites for either muscimol or bicuculline, consistent with previous reports (Laviolette & van der Kooy, 2001; Laviolette *et al.*, 2004).

> 50 ng (of either bicuculline or muscimol) produce seizures and aversive motivational effects, as previously reported (Laviolette & van der Kooy, 2001).

**Both a GABA<sub>A</sub> receptor agonist and antagonist blocked the aversive effects of opiate withdrawal in the ventral tegmental area**

Relative to rats receiving intra-VTA saline, both intra-VTA muscimol (50 ng) and intra-VTA bicuculline (50 ng) completely blocked the

aversive effects of opiate withdrawal, and produced CPP for environments previously paired with either intra-VTA muscimol or bicuculline, even when conditioned in a state of 21-h of opiate withdrawal (Fig. 3C). Two-way ANOVA revealed a significant interaction between group (intra-VTA muscimol, bicuculline or saline) and condition (drug- or saline-paired environments) on times spent in drug or saline vs. neutral, previously unpaired environments ( $F_{2,43} = 24.2, P < 0.05$ ). *Post hoc* analysis revealed that, while saline control rats ( $n = 8$ ) spent significantly less time in the environment paired previously with opiate withdrawal ( $P < 0.05$ ), rats that received intra-VTA bicuculline ( $n = 7$ ) or muscimol ( $n = 7$ ) in a state of opiate

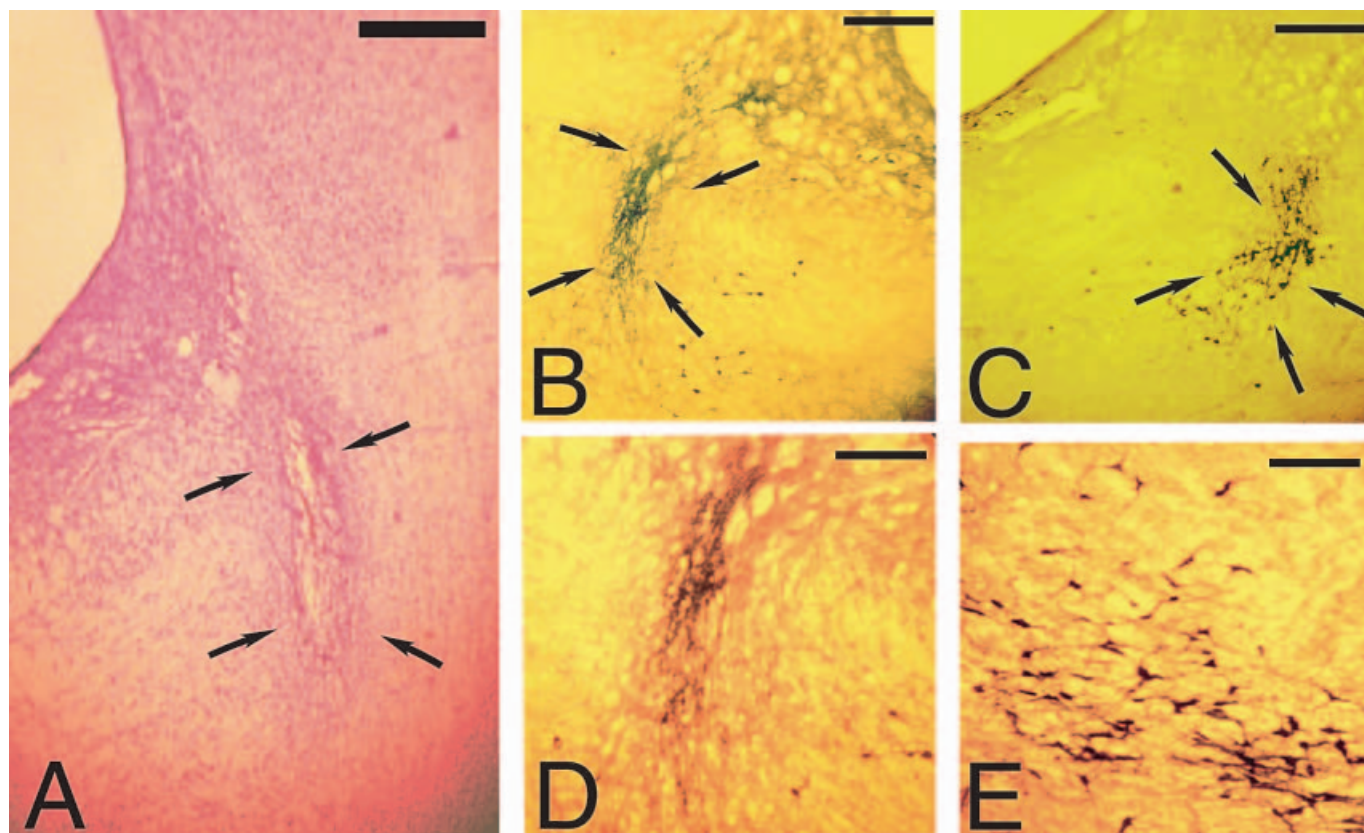


FIG. 2. Histochemical analysis of bilateral TPP lesions. (A) Cresyl Violet analysis revealed substantial gliotic damage that was relatively restricted to the anatomical boundaries of the TPP. Histochemical analysis of NADPH-positive neurons within the TPP revealed significant losses of NADPH-positive neurons within the TPP in (B and D) lesioned relative to (C and E) sham-lesioned rats. Panels D and E are higher magnification photos of panels B and C. Scale bars, 50  $\mu\text{m}$  (A), 40  $\mu\text{m}$  (B and C), 20  $\mu\text{m}$  (D and E).

withdrawal spent significantly greater time in environments paired previously with drug than in the neutral environment (all  $P < 0.05$ ). Thus, intra-VTA muscimol (50 ng) or bicuculline (50 ng) blocked the aversive effects of opiate withdrawal and produced robust preferences for environments paired previously with either muscimol or bicuculline, even when conditioned in a state of 21-h opiate withdrawal.

#### *Lesions of the tegmental pedunclopontine nucleus dissociated GABA<sub>A</sub> receptor-mediated reward signalling in the ventral tegmental area as a function of opiate state*

We next examined the role of the brainstem TPP in the mediation of the rewarding effects of intra-VTA muscimol (50 ng) or bicuculline (50 ng) in rats either previously opiate-naïve or opiate-dependent and in 21 h of withdrawal. Bilateral lesions of the TPP completely blocked the rewarding effects of intra-VTA bicuculline (50 ng) in rats that were conditioned in an opiate-naïve state (Fig. 4A;  $n = 9$ ) relative to sham control rats ( $n = 9$ ). However, TPP lesions had no effect on the rewarding effects of intra-VTA muscimol (50 ng) in previously opiate-naïve rats (Fig. 4B;  $n = 7$ ) relative to sham control rats ( $n = 8$ ). Remarkably, a separate group of rats conditioned in the opiate-dependent and withdrawn state displayed the reverse pattern of results. Thus, in rats conditioned in the opiate-dependent and withdrawn state ( $n = 8$ ), bilateral TPP lesions now had no effect on the rewarding effects of intra-VTA bicuculline (50 ng) relative to sham controls

(Fig. 4A;  $n = 8$ ). However, in opiate-dependent and withdrawn rats, TPP lesions now completely blocked the rewarding properties of intra-VTA muscimol (50 ng; Fig. 4B;  $n = 7$ ) relative to sham controls ( $n = 8$ ). We next examined whether the functional pattern of VTA GABA<sub>A</sub> receptor-mediated reward signalling would be restored to the opiate-naïve state once rats had recovered from opiate dependence. Following the extinction conditioning procedure (see Materials and methods) the rats were retrained in the opiate-naïve state with either bicuculline (50 ng) or muscimol (50 ng), intra-VTA. Extinction tests revealed that place conditioning produced by either bicuculline or muscimol during opiate dependence and withdrawal was completely extinguished and rats displayed no preference for either environment prior to retraining (for bicuculline,  $t_7 = 1.24$ ,  $P > 0.05$ ; for muscimol,  $t_6 = 1.07$ ,  $P > 0.05$ ; Fig. 5A; sham lesioned rats also displayed extinction of the previously CPP for either muscimol or bicuculline; data not shown). However, following retraining with either muscimol (50 ng) or bicuculline (50 ng), intra-VTA, in the opiate-recovered state, intra-VTA muscimol now produced a robust CPP ( $t_6 = 2.53$ ,  $P < 0.05$ ) and the rewarding effects of intra-VTA bicuculline were now completely blocked ( $t_5 = 0.12$ ,  $P > 0.05$ ), similar to the pattern of results observed in previously opiate-naïve rats (Fig. 4A and B). Thus, extinction followed by retraining in the opiate-recovered state revealed that, in the absence of opiate dependence and withdrawal, GABA<sub>A</sub> receptor-mediated bidirectional reward transmission in the VTA reverted to a functionally opiate-naïve state. In Fig. 5C, the effects of bilateral TPP lesions on GABA<sub>A</sub> receptor-mediated reward

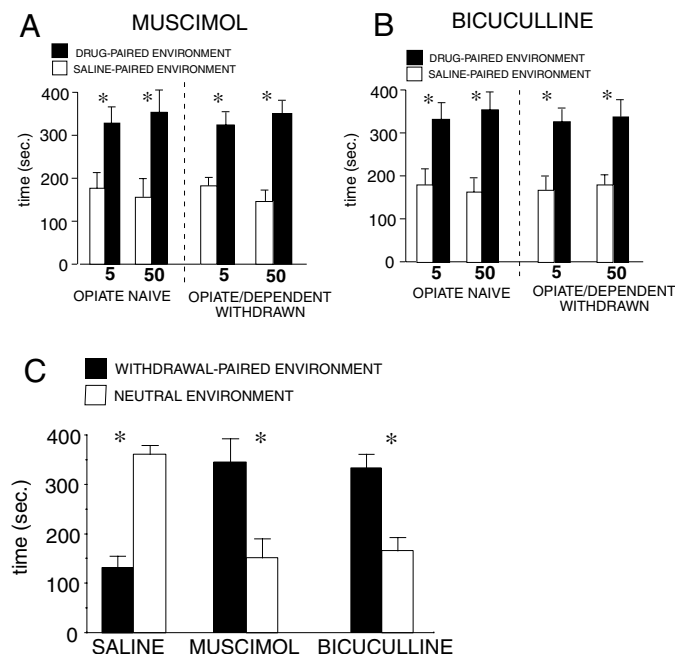


FIG. 3. Motivational effects of intra-VTA muscimol (5 or 50 ng) or intra-VTA bicuculline (5 or 50 ng). (A) Intra-VTA muscimol produced robust rewarding effects over an order-of-magnitude dose range in both opiate-naive and opiate-dependent and withdrawn rats. (B) Intra-VTA bicuculline produced robust rewarding effects over an order-of-magnitude dose range in both opiate-naive and opiate-dependent and withdrawn rats ( $*P < 0.05$ ). (C) In a single-sided conditioned opiate withdrawal procedure, both intra-VTA muscimol (50 ng) and intra-VTA bicuculline (50 ng) blocked the aversive effects of opiate withdrawal and produced significant CPP in opiate-withdrawn rats.

transmission in the VTA are summarized across the opiate-naive, opiate-dependent and withdrawn, extinction, and opiate-recovered phases of training.

## Discussion

In the present study, bilateral lesions of the brainstem TPP revealed a double dissociation for bidirectional GABA<sub>A</sub> receptor-mediated reward signalling in the VTA as a function of opiate state. In rats previously not exposed to opiates, bilateral TPP lesions blocked the rewarding effects of a GABA<sub>A</sub> receptor antagonist but had no effect on the rewarding effects of a GABA<sub>A</sub> receptor agonist in the VTA. However, in a separate group of opiate-dependent and withdrawn rats, TPP lesions blocked the rewarding effects of the agonist while having no effect on the rewarding effects of the antagonist. Once rats had recovered from dependence and withdrawal and the previous reward conditioning was extinguished, GABA<sub>A</sub> receptor-mediated reward signalling in the VTA reverted to the functional parameters observed in rats that had never been exposed to opiates. This suggests that the differential reward signalling between the opiate-naive and opiate-dependent and withdrawn motivational systems can be controlled by the current opiate state.

Some studies have reported that, whereas non-DA neural systems can mediate acute opiate reward, during withdrawal following chronic opiate exposure the mesolimbic DAergic system is activated for the transmission of opiate reward signals (Nader & van der Kooy, 1997; Laviolette *et al.*, 2004). In addition, it has been suggested that, following chronic exposure to psychostimulant

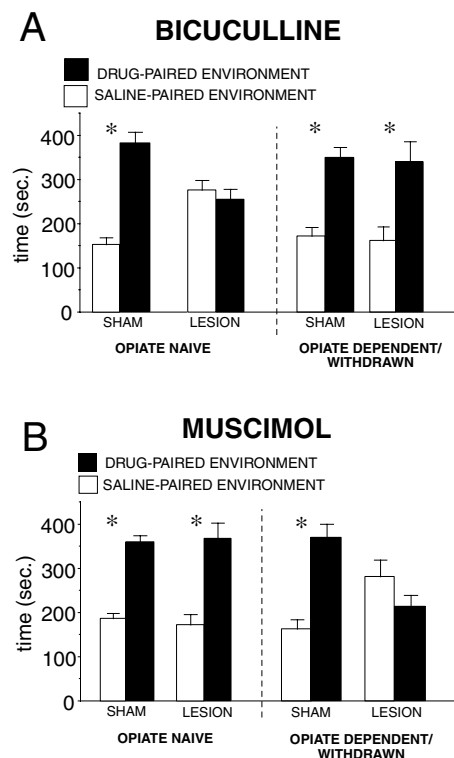


FIG. 4. Effects of TPP lesions on the motivational properties of bicuculline (50 ng) or muscimol (50 ng) in the VTA in either the opiate-naive or opiate-dependent and withdrawn states. (A) Bilateral lesions of the TPP blocked intra-VTA bicuculline reward in opiate-naive rats but not in opiate-dependent and withdrawn rats. (B) In contrast, TPP lesions had no effect on the rewarding properties of intra-VTA muscimol in opiate-naive rats, but completely blocked muscimol reward in opiate-dependent and withdrawn rats.  $*P < 0.05$ .

drugs, neural sensitization processes may lead to heightened drug 'craving' or 'wanting' through the mesolimbic pathway (Berridge & Robinson, 1998; Robinson & Berridge, 1993). However, the present results suggest that the transition between the separate neural systems mediating neural reward signalling in the acute vs. the dependent and withdrawn phases of opiate exposure can be transcended regardless of chronic opiate exposure. Because the neuronal adaptations that have been proposed to account for drug reward sensitization may involve long-lasting molecular adaptations which persist for weeks to months (Kalivas & Stewart, 1991; Spanagel *et al.*, 1993; Vanderschuren *et al.*, 2001; Lu *et al.*, 2003) such a drug sensitization model of opiate motivation is not congruent with the present findings. Indeed, even after chronic opiate exposure, if rats recovered over the course of only 10 days the VTA GABA<sub>A</sub> receptor substrate regulating reward transmission through the TPP was switched back to the functional parameters observed in opiate-naive rats. The present results demonstrate that bidirectional reward transmission through the TPP reward output substrate from VTA GABA<sub>A</sub> receptors is dependent on the current opiate motivational state.

The proposed VTA GABA<sub>A</sub> receptor-mediated bidirectional reward switching mechanism is summarized in Fig. 6. We have reported that bidirectional reward transmission through DA-dependent and DA-independent pathways from the VTA is controlled by the signalling properties of VTA GABA<sub>A</sub> receptors associated with GABAergic neurons (Laviolette & van der Kooy, 2001; Laviolette *et al.*, 2004;



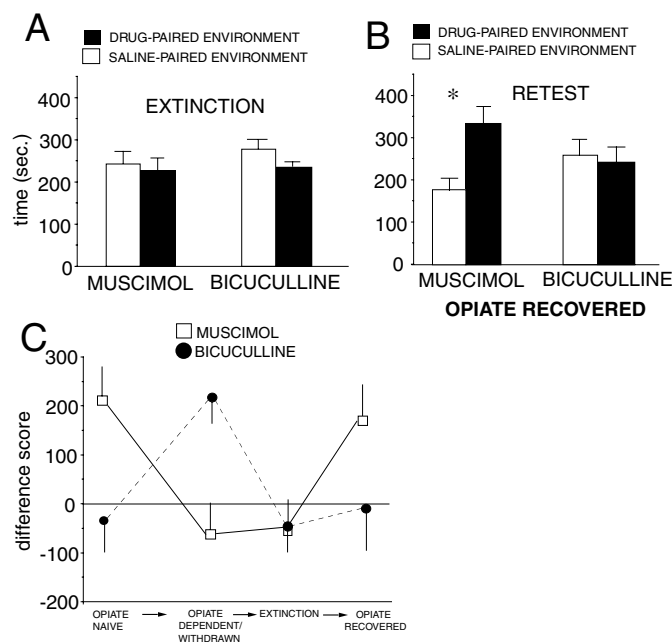


FIG. 5. Effects of extinction conditioning and retraining of intra-VTA muscimol or bicuculline reward in TPP lesioned rats. (A) Lesioned rats, trained previously in the opiate-dependent and withdrawn state then extinguished, showed no preference for either previously conditioned environment at testing. (B) These same rats, retrained with either muscimol (50 ng) or bicuculline (50 ng), intra-VTA, postextinction and after recovery from opiate exposure, demonstrated a blockade of bicuculline reward but not muscimol reward, demonstrating that GABA<sub>A</sub> reward signalling parameters in the VTA revert to the opiate-naive state after a short-term recovery from opiate exposure. (C) A summary figure showing the motivational effects of intra-VTA bicuculline (50 ng) or muscimol (50 ng) in TPP lesioned rats during four different conditioning phases. (1) Opiate-naive: TPP lesions blocked DA-independent bicuculline reward but not muscimol. (2) Opiate-dependent and withdrawn: TPP lesions blocked DA-independent muscimol but not DA-dependent bicuculline reward. (3) Extinction: rats displayed no preference for either previously conditioned environment. (4) Opiate recovered: TPP lesions once again blocked DA-independent bicuculline reward but not DA-dependent muscimol reward. \* $P < 0.05$ .

Fig. 6). Using electrophysiological, pharmacological and immunocytochemical lines of evidence, we have demonstrated that VTA GABA<sub>A</sub> receptors mediate an inhibitory conductance in the opiate-naive state but switch to an excitatory signalling mode in the opiate-dependent and withdrawn state (Laviolette *et al.*, 2004). Accordingly, in the naive state, agonist activation of these receptors inhibits these cells, removing inhibition on the mesolimbic DAergic reward pathway and producing a DA-mediated reward signal whereas antagonist blockade of these receptors increases their activity, producing reward through a non-DAergic substrate (Fig. 6A). However, in the opiate-dependent and withdrawn state, these same GABA<sub>A</sub> receptors switch to an excitatory conductance and agonist activation now increases the activity of these neurons thereby producing DA-independent reward whereas antagonist blockade has the opposite effect, removing inhibition to the DA system and producing DA-dependent reward (Fig. 6B; Laviolette & van der Kooy, 2001; Laviolette *et al.*, 2004). The present results demonstrate that VTA GABA<sub>A</sub> receptors similarly control bidirectional reward signalling through a descending reward output to the TPP as a function of opiate state.

These findings suggest that chronic opiate exposure and dependence alone are not sufficient to induce the switch between these two separate motivational systems: an aversive state of withdrawal from

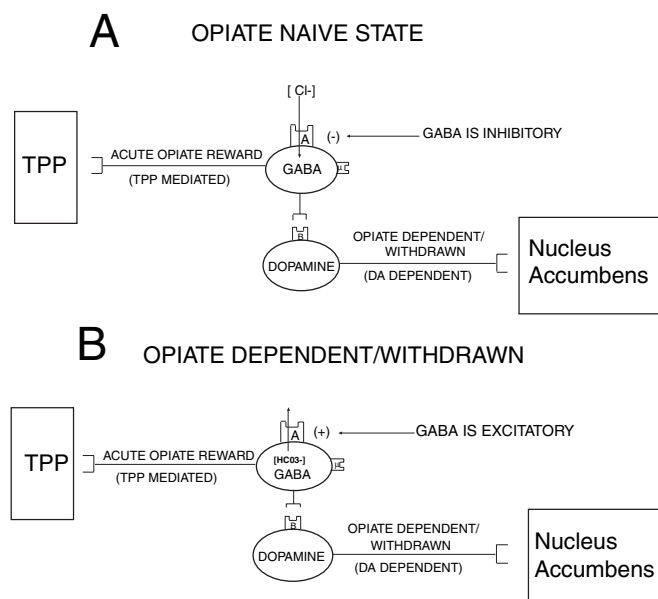


FIG. 6. Schematic model showing the hypothesized GABA<sub>A</sub> receptor-mediated bidirectional reward switching mechanism in the VTA. (A) In the opiate-naive state, GABA<sub>A</sub> receptors associated with VTA GABAergic neurons mediate an inhibitory conductance mediated by Cl<sup>-</sup> influx through the GABA<sub>A</sub> ionophore; thus agonist activation of these receptors inhibits the GABAergic input to the DA system, activating a DA-dependent reward signal. By contrast, antagonist blockade of these receptors increases activity of these neurons and associated efferents, transmitting a DA-independent reward signal to the TPP. (B) In the opiate-dependent and withdrawn state the conductance properties of the GABA<sub>A</sub> receptor switches from inhibitory to excitatory which is dependent upon efflux of HCO<sub>3</sub><sup>-</sup> through the ionophore (Laviolette & van der Kooy, 2004). Now, agonist activation excites the GABAergic neurons, activating DA-independent reward signalling through descending inputs to the TPP reward output, while antagonist blockade decreases activity of these cells, removing inhibition on the DA system and activating the DA-dependent reward signal.

the drug must be present. Dependence upon opiates (in the sense that cessation of the drug will lead to aversive withdrawal) does not in and of itself induce VTA switching between the two neural motivational pathways. Indeed, in the dependent state (but in the absence of withdrawal) rats demonstrated VTA reward signalling parameters that were the same as in rats never previously exposed to opiates. We propose that while the TPP appears critical for the acute rewarding effects of the drug in the early phase of exposure and after recovery from opiate exposure has occurred, a DA-dependent system that is required to signal the rewarding effects of 'withdrawal alleviation' is activated once drug withdrawal is present and the switch between the two systems has been triggered.

Other studies have reported that either bicuculline or muscimol produce robust rewarding effects within the VTA as measured in the ICSA paradigm (Ikemoto *et al.*, 1997; Ikemoto *et al.*, 1998). These studies reported that muscimol produced rewarding effects specifically in the posterior VTA, while picrotoxin or bicuculline only produced reward when self-administered into the anterior VTA. This raises the possibility that the observed functional dissociation between DA-mediated reward signalling and DA-independent reward signalling may be explained by anatomical differences within the VTA itself. In the present and previous reports (Laviolette & van der Kooy, 2001; Laviolette *et al.*, 2004) we have not observed any anatomical differences in the reward efficacy for either bicuculline or muscimol, regardless of whether cannulae tips were in the anterior or posterior limits of the VTA. More importantly, in the present study the same

VTA microinfusion locations (in the posterior and anterior VTA regions) in the same animals produced reward switching between the separate neural motivational systems as a function of opiate state, thereby ruling out anatomical VTA differences as an underlying reason for the observed reward transmission dissociation from the VTA to the TPP. Another study (David *et al.*, 1997) using ICSSA in mice reported that microinfusions of bicuculline into the VTA were attenuated by systemic pretreatment with a D2 receptor antagonist, suggesting that the rewarding effects of intra-VTA bicuculline may be dependent upon DA transmission. In contrast, in the present and previous studies (Laviolette & van der Kooy, 2001, 2004) we have reported that the rewarding effects of intra-VTA bicuculline are DA-dependent only in the opiate-dependent and withdrawn state, not in the acute phase of drug exposure. In the study by David *et al.* (1997), D2 DA receptor antagonism attenuated bicuculline ICSSA in mice that had already learned the procedure but, when the same D2 antagonist was administered in the acute phase, the mice were incapable of learning the discrimination between the drug-paired and non drug-paired associations. Because only bicuculline was tested in this study and no independent control stimulus was used for the effects of the DA antagonist, it is possible that the DA antagonist was producing nonspecific stimulus discrimination effects rather than the motivational effects of bicuculline *per se*. In contrast we report that the same dose of  $\alpha$ -flupenthixol blocks either bicuculline or muscimol reward (but not both at the same time) as a function of opiate state, thereby precluding any nonspecific learning impairments or nonspecific motivational blockade. Future studies are required to address these discrepancies.

If the development of drug dependence and withdrawal is considered a progressive transition from nondependence to a static state of addiction, once dependence has developed, theories stressing long-lasting neuroadaptations as underlying causes of drug dependence (Berridge & Robinson, 1998; Koob & Le Moal, 2001; Ahmed *et al.*, 2002; Pecina *et al.*, 2003) would not necessarily predict the GABA<sub>A</sub> receptor-mediated VTA functional switching effects through the TPP observed in the present study. (Fig. 5C). Indeed, if a more permanent alteration in drug reward circuitry was induced by chronic heroin exposure, the same TPP lesions would have probably produced the same pattern of GABA<sub>A</sub> reward blockade as that observed when rats were conditioned in withdrawal. Instead, the VTA GABA<sub>A</sub> receptor substrate switched back to the functional parameters observed in rats not previously exposed to opiates. This occurred after a short recovery period, when other opiate sensitization phenomena, such as motor sensitization, are still present (Leite-Morris *et al.*, 2004; Vanderschuren *et al.*, 2001). While our findings do not preclude the possibility that a progressive sensitization of DA pathways may underlie increased drug craving and 'wanting' as the addiction cycle progresses, the observed switching between the two neural motivational systems (Fig. 6) takes place over the course of chronic drug exposure. In other words, even in a state of dependence (but in the absence of withdrawal), VTA reward signalling would still be mediated through a non-DA reward system, just as in the acute state.

Interestingly, it has been reported that relapse to heroin self-administration in rats is dependent upon DA transmission (Shaham & Stewart, 1996). Thus, even though rats were extinguished from heroin self-administration and recovered from opiate exposure the neural mechanism triggering relapse to heroin self-administration was DA-dependent. Although we did not examine relapse in the present study, this finding may suggest that the neural mechanism(s) responsible for drug relapse may be separate from the hypothesized VTA switching mechanism (Fig. 6). Alternatively, because these rats had learned the

cues for heroin reward in an opiate-dependent and withdrawn state, these same cues could conceivably still control motivation based on reactivating the reward-associated cues learned during the dependent and withdrawn opiate state. Thus, in the drug relapse paradigm, if the rewarding effects of opiates were learned in the dependent and withdrawn state then stress or heroin-induced reactivation of those cues may still be DA-dependent and thus blocked by neuroleptics at testing (Shaham & Stewart, 1996).

In summary, we report that both bicuculline and muscimol in the VTA produce acute rewarding effects and can block the aversive effects of opiate withdrawal. Our results suggest that the brainstem TPP serves as a DA-independent reward output substrate for bidirectional GABA<sub>A</sub> VTA reward signalling as a function of opiate state. Similarly to ascending mesolimbic DA reward signalling from the VTA (Laviolette & van der Kooy, 2004), this descending reward pathway to the TPP from the VTA is controlled by a molecular switch occurring at the GABA<sub>A</sub> receptor that gates bidirectional reward transmission as a function of opiate state.

## Abbreviations

CPP, conditioned place preference; ICSSA, intracranial drug self-administration; TPP, tegmental pedunculopontine nucleus; VTA, ventral tegmental area.

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