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Tegmental Pedunculopontine Glutamate and GABA-B Synapses Mediate Morphine Reward

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The tegmental pedunculopontine nucleus (TPP) of the midbrain is critical in mediating the acute rewarding effects of opiates. However, the circuitry and neurochemistry underlying this effect has not been determined. Here we identify TPP receptors and cell types involved in systemic morphine reward and suggest an anatomical and neurochemical model for reward in the TPP. Simple hypothetical anatomical models for serial cell arrangements and receptors in the TPP were proposed and predictions of behavioral outcome (reward or no reward) then were made, based on the administration of agonists and antagonists directly into the TPP of rats. We report that TPP-administered NMDA produced rewarding effects, although GABA agonists and antagonists had no motivational effects on their own. However, the NMDA receptor antagonist AP-7 and the GABA-B receptor antagonist saclofen, while having no motivational effects on their own, blocked systemic morphine reward as measured by conditioned place preference. These results provide positive evidence for GABA-B and glutamate synapses in the TPP, which mediates systemic morphine reward and suggest that a serial pathway for morphine reward in the TPP is unlikely.

Keywords: reward, conditioned place preference, neuroanatomy, morphine, drug addiction

The brainstem tegmental pedunculopontine nucleus (TPP), composed of the much-studied Ch5 cholinergic cell group as well as noncholinergic perikarya such as glutamate (Clements & Grant, 1990) and GABA (Bevan & Bolam, 1995; Ford, Holmes, Mainville, & Jones, 1995) neurons, has been shown to have a role in reward and motivation (Alderson & Winn, 2005; Bechara & van der Kooy, 1989; Lepore & Franklin, 1996; Morgenson, Jones, & Yim, 1980; Olmstead & Franklin, 1993; Yeomans, Mathur, & Tampakeras, 1993). Lesions of the TPP block the rewarding effects of opiates (Bechara & van der Kooy, 1992; Olmstead & Franklin, 1993; Olmstead, Munn, Franklin, & Wise, 1998), and also brain self-stimulation (Lepore & Franklin, 1996) and nicotine (Laviolette, Alexson, & Van der Kooy, 2002).

Despite these findings, little is known about the circuitry and neurochemistry behind these rewarding effects in the TPP. The goal of this study was to identify receptors and cell types involved in morphine reward and suggest an anatomical and neurochemical model for reward in the TPP based on these data.

NMDA receptor-bearing cells are thought to be critically involved in reward in the TPP because excitotoxic NMDA lesions of the TPP block the effects of rewarding stimuli (Bechara & van der Kooy, 1989; Olmstead & Franklin, 1993). Morphine microinfused into the ventral tegmental area (VTA) produces potent rewarding effects (Blas-Kubik, Ableitner, Herz, & Shippenberg, 1993; van der Kooy, Mucha, O'Shaughnessy, & Bucenieks, 1982) and opiate receptor-bearing VTA GABA cells send projections to the TPP (Semba & Fibiger, 1992; Steiniger-Brach & Kretschmer, 2003, 2005; Swanson, 1982). Thus, the VTA has been considered in this study as a likely candidate for GABA input to the TPP in the mediation of morphine reward, though there are other TPP GABA inputs that may be more dense than those from the VTA (Mena-Segovia, Bolam, & Magill, 2004). Also, there is some evidence that VTA GABA cells may also contain glutamate (Sulzer et al., 1998) and colocalization of GABA and glutamate is seen in cells in other brain regions (Sandler & Smith, 1991; Somogyi & Llewellyn-Smith, 2001). It is therefore possible that a VTA GABA projection to the TPP also releases glutamate to mediate morphine's motivational effects, though other glutamate inputs to the TPP are not ruled out.

Based on the neural connections mentioned above and on the significant numbers of glutamate, GABA, and acetylcholine neuronal perikarya in the TPP (Lavoie & Parent, 1994), we proposed several simple anatomical models and made behavioral predictions (reward or nonreward) for each of these hypothetical models with respect to the agonists and antagonists to be microinfused into the TPP (see Figure 1). The effect of these intra-TPP drugs on morphine CPP were also predicted. For the sake of simplicity, a limit of two cells or fewer, connected in a serial fashion was set. Also, because muscarinic ACh receptors may be present on non-ACh cells within the TPP (Luebke, McCarley, & Greene, 1993), and may therefore have a role in the transmission of a reward signal through the TPP, we examined the effect of a TPP-administered muscarinic acetylcholine (mACh) receptor antagonist on systemic morphine reward.

We tested rats in the place conditioning procedure under the influence of different GABA and glutamate agonists and antago-

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nists administered into the TPP, and challenged the morphine place preference with these intra-TPP drugs as well as a mACh receptor antagonist. To assess possible nonspecific effects the intra-TPP drugs might have on the sensation of cues, learning, and memory, and the ability to make the appropriate response, we tested animals with a naloxone place aversion.

Materials and Method

Surgery and Histology

All procedures were in accordance with an Animal Use Protocol (No. 20006625) approved by the University Animal Care Committee, which complies fully with requirements under Ontario's Animals for Research Act and the federal Canadian Council on Animal Care.

Male Wistar rats (Charles River Canada, St. Constant, Quebec) were anesthetized with inhaled isoflurane (5% to induce anesthesia and 1–3% to maintain anesthesia) and placed in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA). 22-gauge stainless steel guide cannulas cut 9 mm below the pedestal (Plastics One, Roanoke, VA) and angled 10° toward the midline were inserted so that injectors (stainless steel 28-gauge, extending 2 mm beyond the guide cannula, Plastics One) used with the guide cannulas were aimed at the TPP 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 the end of behavioral experiments, animals were deeply anesthetized with a lethal dose of sodium

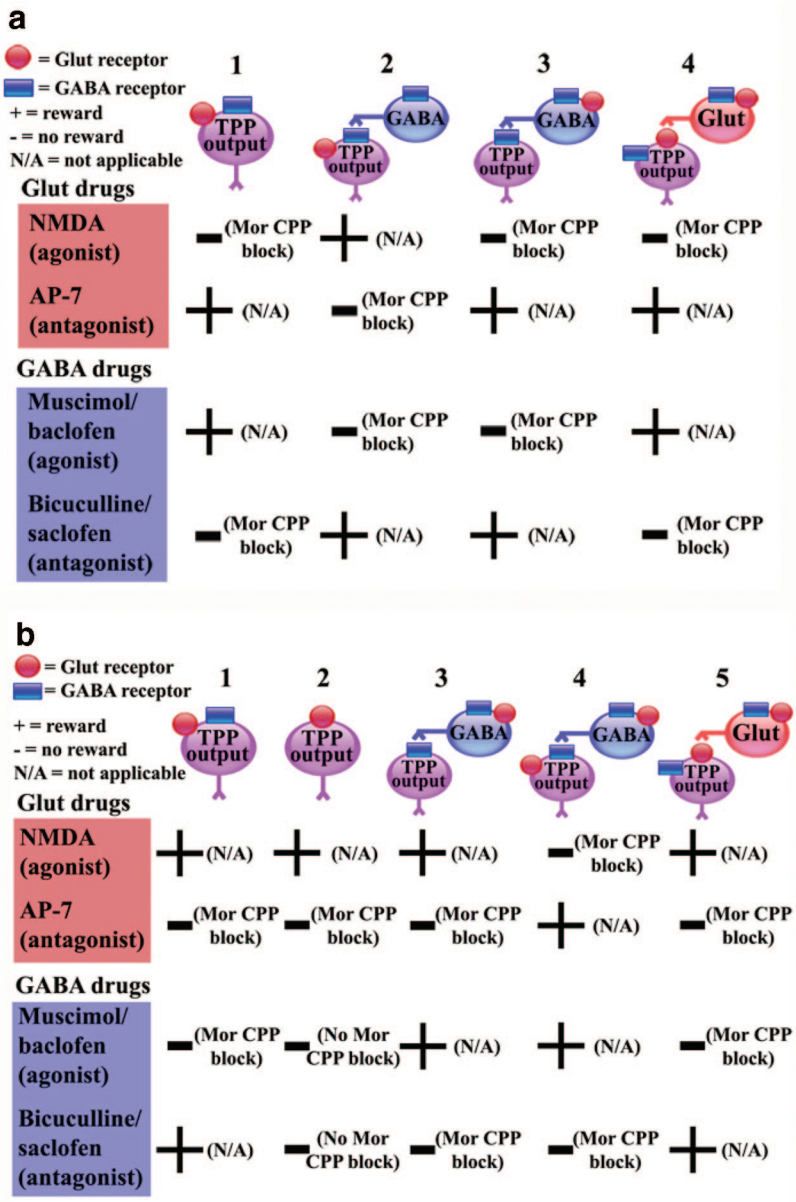

Figure 1. (opposite).

pentobarbital (35 mg/kg) and perfused intracardially with approximately 100 ml of 0.9% saline followed by approximately 200 ml of a 10% formalin solution. Brain sections were stained with cresyl violet, and cannula placements were verified with light microscopy. Animals with cannula placements outside the borders of the TPP as well as animals found to have extensive, bilateral damage to the TPP were excluded from analysis. The verification of cannula position was done by an experimenter unaware of the experimental treatment and outcome for each animal.

Drugs and Microinfusion Procedure

The drugs used in these experiments were morphine sulfate (Almat Pharmachem Inc., Concord, Ontario), bicuculline methiodide (Sigma Chemical, St. Louis, MO), N-Methyl-D-aspartic acid (NMDA; Sigma), D(-)-2-Amino-7-phosphono-hepatanoic acid (AP-7; Sigma), saclofen (Sigma), baclofen (Sigma), scopolamine (Sigma), and naloxone HCl (Sigma).

For GABA and glutamate experiments, a systemic morphine dose of 5 mg/kg was chosen based on previous results showing a significant and reliable place preference at this dose, without suffering from ceiling effects and making the preferences relatively insensitive to disruption. A systemic morphine dose of 10 mg/kg was used for the scopolamine experiment. Bilateral microinfusions (volume of 0.5 μl per infusion) were performed over 1 min and left in place for an additional 1 min postinfusion to allow for spread of the drug from the injector tip. Animals were then immediately placed in the appropriate conditioning environment.

Place Conditioning

All animals were conditioned using a fully counterbalanced place-conditioning procedure (Nader & van der Kooy, 1997). Conditioning took place in one of two distinct environments. One box was black with a black Plexiglas floor, the other was white with an aluminum floor over which was placed a 1 cm wire grid

Figure 1a. Anatomical circuit scenarios: GABA from VTA. A table describing behavioral predictions made based on different possible anatomical circuit models in the TPP. These predictions are made based on the assumption that systemic morphine causes GABA to be released from VTA GABA cells that project to a single cell type in the TPP. The left side of the figure lists the different drugs to be infused into the TPP immediately prior to conditioning. NMDA and GABA-B receptors were stimulated with NMDA and baclofen, respectively. NMDA, GABA-A and GABA-B receptors were antagonized with AP-7, bicuculline, and saclofen, respectively. The top of the figure shows schematic diagrams of four anatomical models. In the first, a single TPP output cell having both a glutamate and GABA receptor is stimulated by GABA from VTA GABA neurons in the systemic morphine condition, and the resulting inhibition of this TPP output cell yields reward. Therefore, any stimulus that inhibits this TPP output cell will similarly lead to reward (denoted as "+"), such as the application of a GABA agonist or a glutamate antagonist. Any stimulus that excites the cell should not lead to reward (nonreward denoted as "–"), such as the application of NMDA or bicuculline/saclofen. In the case that a TPP-infused drug has no rewarding effect, its effect on systemic morphine reward (either a block of morphine CPP or no block of morphine CPP) can be predicted. Any drug that opposes the inhibitory action on the TPP cell caused by systemic morphine should block the morphine place preference, as would be the case with NMDA and bicuculline/saclofen. The second scenario involves two cells, the first a GABA cell lacking a glutamate receptor that projects to an output cell having both a GABA and glutamate receptor. In this case, drugs applied to the TPP affect both cells, with the action of the output cell dictating the behavioral effect. Systemic morphine causes inhibition of the upstream GABA cell resulting in a disinhibition of the output cell. Therefore, any drug that disinhibits or excites the downstream cell will lead to reward and any drug that inhibits the downstream cell will block systemic morphine reward. The third scenario is identical to the second with the exception that the first cell in the chain, the GABA cell, has a glutamate receptor whereas the output cell to which it projects does not. The fourth scenario involves a glutamate cell that projects to an output cell, with both cells having a glutamate and a GABA receptor. In this case, systemic morphine results in the inhibition of the glutamate cell that reduces the tonic stimulation of the downstream output cell. Any drug that reduces the activity of the downstream cell will result in reward whereas any excitation of this cell will block systemic morphine reward. N/A indicates that a morphine CPP block experiment would not be useful in cases in which the intracranial drug on its own is rewarding because results would be difficult to interpret owing to the fact that both stimuli are rewarding. The scenario involving a single output cell with only a GABA receptor has been omitted because past NMDA lesion experiments showing a block of morphine reward necessitate a NMDA receptor in the TPP for morphine reward. *Figure 1b:* Anatomical circuit scenarios: Glutamate from VTA. A table describing behavioral predictions made based on different possible anatomical circuit models in the TPP. These predictions are made based on the assumption that systemic morphine causes glutamate to be released from VTA GABA cells that project to a single cell type in the TPP. The left side of the figure lists the different drugs to be infused into the TPP immediately prior to conditioning. NMDA and GABA-B receptors were stimulated with NMDA and baclofen, respectively. NMDA, GABA-A and GABA-B receptors were antagonized with AP-7, bicuculline and saclofen, respectively. The top of the figure shows schematic diagrams of five anatomical models. In the first, a single output cell having both a glutamate and GABA receptor is stimulated by glutamate from VTA GABA neurons in the systemic morphine condition, and the excitation of this TPP output cell results in reward. Therefore, any stimulus that excites this output cell will similarly lead to reward (denoted as "+"), such as the application of a glutamate agonist or a GABA antagonist. Any stimulus that inhibits the cell should not lead to reward (nonreward denoted as "–"), such as the application of AP-7 or baclofen. In the case that a TPP-infused drug has no rewarding effect, its effect on systemic morphine reward (either a block of morphine CPP or no block of morphine CPP) can be predicted. Any drug that opposes the excitatory action on the TPP cell caused by systemic morphine should block the morphine place preference, as would be the case with AP-7 and baclofen. The second scenario involves a single output cell having only a glutamate receptor. The third scenario involves two cells, the first a GABA cell that projects to an output cell, with both cells having a GABA receptor, but only the GABA cell having a glutamate receptor. In the fourth scenario, the output cell has a glutamate receptor. This setup is analogous to scenario two in Figure 1a, except that it requires a glutamate receptor on the first (GABA) cell because glutamate is proposed to be released onto it from the VTA cell. These two schemes involve the inhibition of the output cell by the upstream cell's release of GABA in the systemic morphine condition. They differ in the effect the application of intra-TPP glutamate has on its own and its effect on systemic morphine reward. The fifth scenario involves a glutamate cell that projects to an output cell, both having a glutamate and a GABA receptor. In the systemic morphine condition, the output cell is stimulated by the upstream cell's release of glutamate. N/A indicates that a morphine CPP block experiment would not be useful in cases in which the intracranial drug on its own is rewarding because results would be difficult to interpret owing to the fact that both stimuli are rewarding.

floor. A solution of 2% acetic acid was wiped onto the black Plexiglas just prior to placing an animal into the black box. These environments are motivationally balanced so that animals show no initial preference for either environment (data not shown).

All experiments used a fully counterbalanced place conditioning procedure. In this procedure, the animals were given four, six, or eight drug administrations spaced over 8, 12 or 16 days, depending on the experiment, and given an equal number of vehicle administrations on alternate days. Conditioning sessions each lasted 40 min.

When both an infusion and an injection were used in an experiment, as in experiments involving an intracranial drug and morphine or naloxone, the animal was given the intracranial drug every day immediately followed by either the intraperitoneally (ip) injected drug or vehicle according to the conditioning schedule, before being placed in the appropriate conditioning environment. The intracranial drug was administered in both drug and vehicle trials in order to balance its effects equally over both drug and vehicle trials. In experiments using ip-injected naloxone, animals were conditioned for 8 days using a naloxone dose of 5 mg/kg and tested as described below. At least 1 day after testing took place, animals were conditioned for an additional 4 days using the higher dose of naloxone (10 mg/kg) and tested subsequently. In all other experiments, animals were conditioned for 8 days and tested with no subsequent conditioning.

Subjects in the experimental groups saclofen, saclofen + morphine, AP-7 (4 μg/side), and one group of AP-7 (2 mg/side) were reassigned to new experimental groups naloxone control, saclofen + naloxone and AP-7 + naloxone so that each new experimental group had at least two members from each of the previous experimental groups, and were fully counterbalanced for treatment and for the environment with which the drug was paired, taking into account their previous treatment.

Testing was performed drug-free, at least 2 days after the final conditioning session by an experimenter blind to the treatment group as well as to the treatment each animal received within a group. Animals were placed in a narrow gray area separating the two test compartments and allowed to move freely between each environment within the test box for 10 min and time spent in each of the two compartments of the test box was counted. The time an animal spent in a given compartment was measured with a timer according to visual observation by an experimenter. The time spent in the previously drug-paired environment was compared with the time spent in the previously vehicle-paired environment and the preference for or avoidance of the drug-paired environment was taken as a measure of that drug's motivational effect (van der Kooy, 1987).

Statistics

For each experimental group, a paired two sample *t* test for means was used to determine if the average time spent in the drug-paired environment and the average time spent in the vehicle-paired environment during the test were significantly different. For procedures involving multiple-drug treatments and/or doses, one- or two-way analyses of variances (ANOVAs) were performed as necessary. A Newman–Keuls post hoc test was conducted as needed.

Results

Histology

Histological analysis of intra-TPP cannula placements revealed that of a total of 132 animals that underwent cannula implantation surgery, 36 were excluded from analysis because either their cannula tips were outside the boundaries of the TPP as defined by Paxinos and Watson (Paxinos & Watson, 1986), or they had large unilateral or bilateral lesions of the TPP due to infection or cannula damage. The remaining animals had appropriate cannula placements within the TPP and were included in the analyses.

Effects of GABA Agonists and Antagonists in the TPP

Administration of the GABA-A receptor antagonist bicuculline (25 ng/side) into the TPP produced hyperlocomotion and turning after the animal was placed in the conditioning environment. This dose of intracranial bicuculline was used because previous experiments (not shown) revealed that doses higher than this caused violent hyperlocomotion. Also, the dose of bicuculline used here matched that used in experiments involving microinfusions into the VTA, which produced robust behavioral effects (Laviolette & van der Kooy, 2001).

There was no significant difference between the time spent in the drug-paired and vehicle-paired environments during the test, $t(6) = -0.6$, $p > .05$ (see Figure 2). Thus, bicuculline failed to produce any significant motivational effects when administered into the TPP.

The effects of GABA-B receptor blockade in the TPP were examined next. A dose of 4 μg per side of the GABA-B receptor

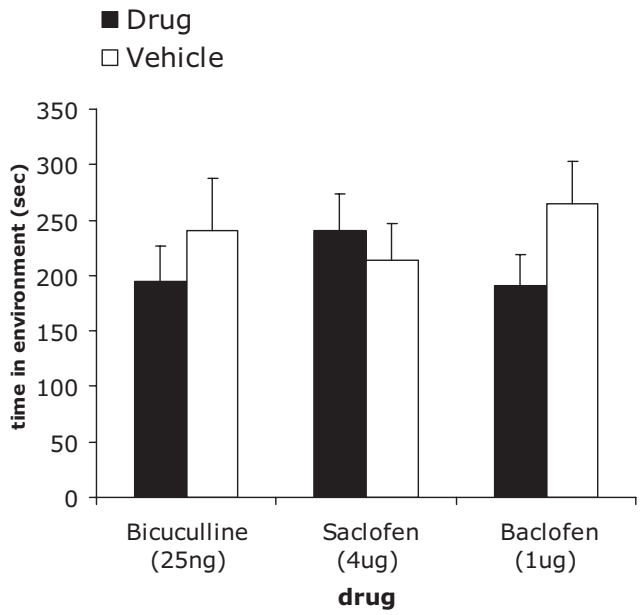

Figure 2. Motivational effects of intra-TPP GABA receptor agonists and antagonists. Bars represent $M \pm SEM$. Animals showed no significant place preferences for intra-TPP bicuculline ($n = 7$), saclofen ($n = 10$), or baclofen ($n = 8$). $ps > .05$. Seconds ± *SEM*.

antagonist saclofen was chosen based on the ability of this drug at doses of 1.5 to 3 μg, to alter opioid-agonist-induced feeding when administered into the NAc and VTA (Ackerman, Lamonte, & Bodnar, 2003). This higher dose (4 μg) was used to account for possible differences in receptor distributions between the VTA/NAc and TPP and to ensure that GABA-B receptors were fully blocked.

Animals conditioned with intra-TPP saclofen spent approximately equal amounts of time in the drug-paired and vehicle-paired environments during the test, $t(9) = 0.4$, $p > .05$ (see Figure 2). Thus, blockade of GABA-B receptors in the TPP had no motivational effects.

To explore whether a GABA-B receptor agonist could produce gain-of-function effects, baclofen was put into the TPP. A dose of 1 μg per side was chosen based on a study examining the effect of 0.5 to 2 μg of baclofen per rat (administered into the dorsal hippocampus) on morphine place preferences (Zarrindast, Massoudi, Sepehri, & Rezayof, 2006). At 1 μg baclofen, animals displayed reduced locomotion and a reduced response to being handled when being removed from the conditioning box. Testing after this latest conditioning phase revealed a slight, nonsignificant aversion, $t(5) = 1.1$, $p > .05$ (see Figure 2). Thus, stimulating GABA-B receptors in the TPP had no significant motivational effects.

Effects of Glutamate Agonists and Antagonists in the TPP

Because glutamate receptors are present in the TPP (Winn, 2006), the possibility that glutamate could be released in the TPP from VTA GABA neurons or from interneurons in the TPP was examined by putting a nonexcitotoxic dose of NMDA, a glutamate receptor agonist, into the TPP. A dose of 75 ng per side was chosen based on the upper range used in experiments administering NMDA into the VTA to produce reinforcing effects and hyperactivity (Ikemoto, 2004). This dose produced slightly increased locomotion. It also was sufficient to produce a significant preference for the drug-paired environment, $t(5) = 4.1$, $p < .05$ (see Figure 3). Thus, NMDA is rewarding when administered into the TPP. Administration of the NMDA receptor antagonist AP-7 at all doses used produced circling behavior and hyperlocomotion in most of the animals receiving the drug. Animals were conditioned first with a dose of 2 μg per side, and showed a very slight, but nonsignificant aversion to the drug, $t(15) = -1.3$, $p > .05$. This dose was chosen based on intra-NAc infusion of this drug at a dose of 1 μg per side in rats and its effect on performance of a passive avoidance task (Martinez et al., 2002). To explore the possibility that the dose was not high enough to produce motivational effects, another group of animals was conditioned at an intra-TPP dose of 4 μg per side. This group showed approximately equal preferences for both the drug-paired and the saline-paired environments, $t(6) = 0.4$, $p > .05$ (see Figure 3). Therefore, blockade of NMDA receptors in the TPP produced no motivational effects at the relatively high doses used. A two-way ANOVA showed no significant differences between the two doses of AP-7 used, $F(1, 45) = 0.018$, $p > .05$, nor any Drug × Dose interaction, $F(1, 45) = 1.92$, $p > .05$.

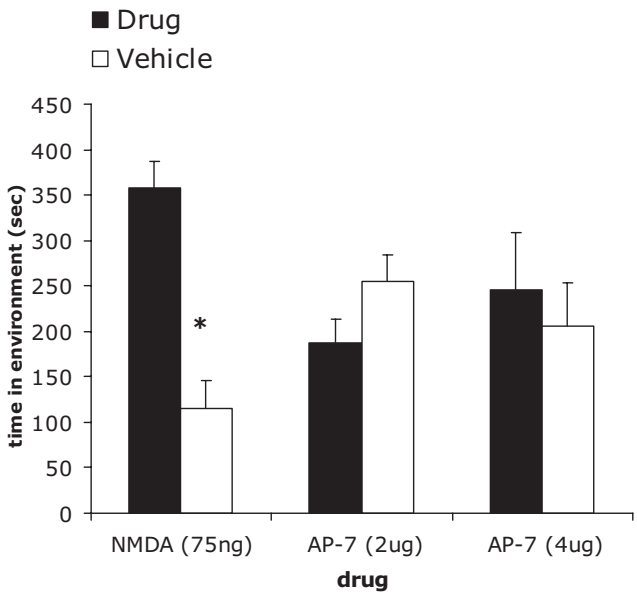

Figure 3. Motivational effects of intra-TPP NMDA receptor agonists and antagonists. Bars represent $M \pm SEM$. Animals given intra-TPP NMDA ($n = 7$) showed a significant preference for the environment previously paired with that drug ($p < .05$). The NMDA receptor antagonist AP-7 at two separate doses (2 μg/side [$n = 16$] and 4 μg/side [$n = 8$]) failed to produce any significant motivational effects when administered to the TPP. ps $> .05$. Seconds ± *SEM*. A two-way ANOVA failed to reveal any significant differences between the two doses of AP-7 ($p > .05$).

Effects of GABA, Glutamate and mACh Receptor Antagonists on Systemic Morphine Place Preference

Animals were administered intra-TPP PBS, bicuculline (a GABA-A receptor blocker), saclofen (a GABA-B receptor blocker), or AP-7 (mACh receptor blocker) to test whether any treatment affected the preference produced by 5 mg/kg systemic morphine (see Figure 4). A two way ANOVA revealed a main effect of drug (morphine vs. saline), $F(2, 65) = 5.88$, $p < .05$, and a significant interaction between Drug × Treatment (PBS vs. bicuculline vs. saclofen vs. AP-7), $F(6, 65) = 2.80$, $p < .05$. A Newman–Keuls post hoc test revealed that intra-VTA PBS or bicuculline had no effect on the 5 mg/kg morphine conditioned place preference ($p < .05$). In contrast, intra-VTA administration of saclofen and the low dose of AP-7 attenuated the 5 mg/kg morphine conditioned place preference ($p > .05$). Therefore, systemic morphine reward is not disrupted by blockade of GABA-A receptors in the TPP, but is disrupted by blockade of GABA-B or NMDA receptors in the TPP.

Morphine, at a dose of 10 mg/kg also produced a significant place preference in animals receiving sham (PBS) intra-TPP infusions, $t(7) = 2.36$, $p < .05$ (see Figure 5). Scopolamine, a mACh receptor antagonist failed to block the morphine place preference at 10 mg/kg, with animals showing a significant preference for the morphine-paired side, $t(8) = 3.85$, $p < .05$ (see Figure 5).

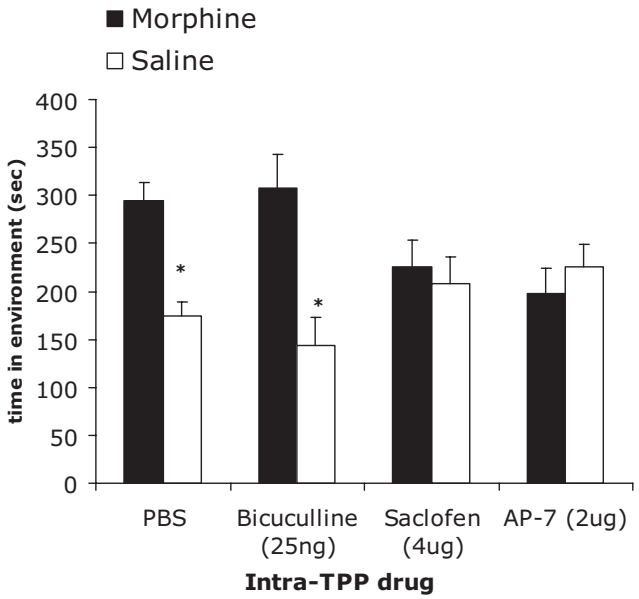

Figure 4. Effects of intra-TPP GABA and glutamate drugs on the morphine conditioned place preference. Bars represent $M \pm SEM$. Animals showed a significant place preference for the environment previously paired with morphine when given a sham (PBS) intra-TPP infusion. Animals given intra-TPP bicuculline also expressed the morphine conditioned place preference. Animals given saclofen or AP-7 showed no preference for the morphine-paired environment. Seconds $\pm$ *SEM*.

Effects of Antagonists on Systemic Naloxone Aversion: Behavioral Controls

Naloxone, an opiate receptor antagonist, has been used to precipitate a state of opiate withdrawal in opiate dependent animals (Bechara, Nader, & van der Kooy, 1995) and has been shown to be aversive when administered systemically to both opiate naive (Mucha, van der Kooy, O'Shaughnessy, & Bucenieks, 1982) and opiate dependent animals (Hand, Koob, Stinus, & Le Moal, 1988). More important, TPP lesions do not block naloxone place aversions (Bechara et al., 1995), indicating that the aversive effect is mediated independently of this nucleus. Naloxone was used in this experiment as a control to determine if the GABA-B and NMDA receptor antagonists were simply preventing the animals from sensing or remembering the environmental cues, learning the appropriate response, or making a response on the test day rather than specifically blocking systemic morphine reward. If the antagonists have an effect on any of the former, the morphine place preference would not be manifested because though the animals would be capable of experiencing reward, they would ultimately be unable to express a place preference on the test day. If the antagonists genuinely block morphine reward specifically, then the aversive effects of systemic naloxone should manifest themselves in place conditioning.

Animals that received intraperitoneal naloxone alone at a dose of 5 mg/kg for 8 conditioning days along with intra-TPP PBS showed an aversion to the naloxone-paired environment compared to the saline-paired environment. However, this aversion was not significant, $t(8) = -0.8$, $p > .05$ (data not shown). The dose was increased to 10 mg/kg and the same animals were conditioned for an additional 4 days in an attempt to produce a significant aversion. This dose of naloxone may not be specific to the blockade of opiate receptors, however because this drug is being used to test for nonspecific sensory, learning, or response effects, not the blockade of opiate effects, the effect it may have on nonopiate receptors is of less concern in this study. Testing after this period revealed a significant aversion to the naloxone paired environment, $t(8) = -2.4$, $p < .05$ (see Figure 6). The results obtained with the two doses were compared using a one-way ANOVA that revealed a significant difference between the means of the two groups, $F(1, 15) = 5.37$, $p > .05$ (data not shown).

Because the naloxone control alone and the antagonist plus naloxone groups were run concurrently, a procedure identical to that of the naloxone alone control was followed. Animals were treated with saclofen (4 μg/side) or AP-7 (2 μg/side) every day during naloxone place conditioning. A two-way ANOVA revealed a main effect of naloxone, $F(1, 45) = 38.2$, $p < .05$, indicating that blockade of GABA-B or NMDA receptors failed to block systemic naloxone place aversions.

Discussion

Although the GABA-A, GABA-B, and glutamate receptor antagonists failed to produce motivational effects on their own, the GABA-B and glutamate receptor antagonists completely blocked systemic morphine place preferences, the mACh receptor and GABA-A antagonists failed to do so. This speaks to the pharmacological specificity of the GABA-B and glutamate antagonist drugs in the TPP, in that they only have an effect on the motiva-

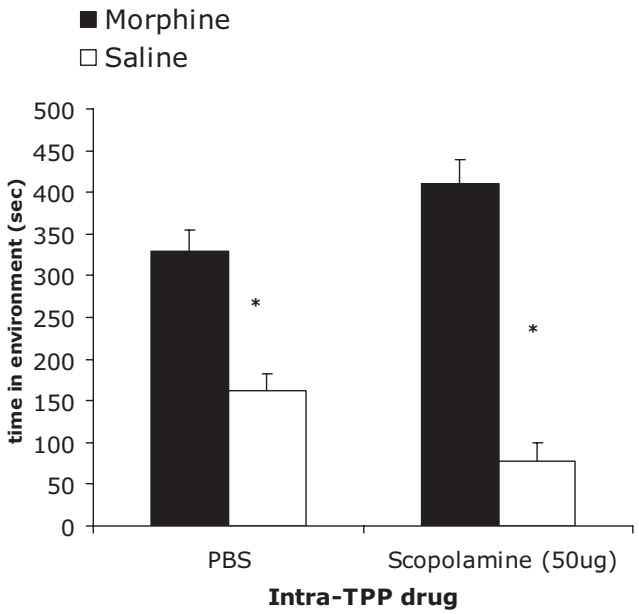

Figure 5. Effects of intra-TPP mACh receptor antagonist on the morphine conditioned place preference. Bars represent $M \pm SEM$. Animals showed a significant place preference for the environment previously paired with 10 mg/kg systemic morphine when given a sham (PBS) intra-TPP infusion ($n = 8$, $p < .05$). Animals given intra-TPP scopolamine also showed a significant preference for the morphine-paired side ($n = 9$, $p < .05$). Seconds $\pm$ *SEM*.

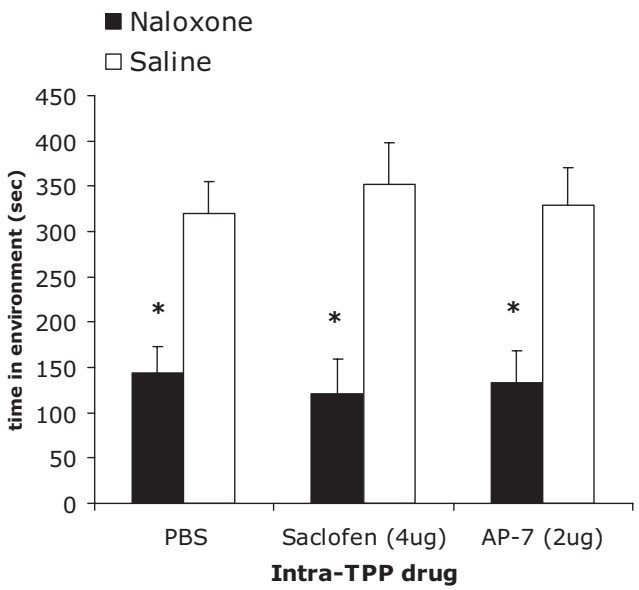

Figure 6. Effects of an intra-TPP GABA-B receptor antagonist and an intra-TPP NMDA receptor antagonist on the naloxone-conditioned place aversion. Bars represent $M \pm SEM$. Animals showed a significant place aversion to environments previously paired with naloxone when given intra-TPP PBS, saclofen, or AP-7. Seconds ± *SEM*.

tional properties of morphine and none on their own. The glutamate agonist alone produced a significant place preference although the GABA-B agonist failed to do so.

These results indicate that both glutamate and GABA receptors are involved in systemic morphine reward in the TPP because their blockade prevents morphine reward. Though this study focused on TPP afferents from the VTA as the source of GABA or glutamate inputs, other sources of glutamate to the TPP include the cortex (Matsumura et al., 2000) and the subthalamic nucleus (Hammond, Rouzaire-Dubois, Féger, Jackson, & Crossman, 1983) and sources of GABA include the globus pallidus, ventral pallidum, substantia nigra, and ventral striatum (Grofova & Zhou, 1998; Semba & Fibiger, 1992; Steiniger-Brach & Kretschmer, 2003). Indeed, some of these other inputs may be denser than those from the VTA and may play a role in mediating opiate reward in the TPP. However, considering that u-opiate receptors in the VTA are predominantly located on GABA cells (Dilts & Kalivas, 1989; Mansour, Lewis, Khachaturian, Akil, & Watson, 1986), which are acted on by opiates administered there (Johnson & North, 1992; Steffensen et al., 2006), we can be confident that targets of the descending, nondopamine (DA) GABA projections from the VTA will have GABA receptors and likely play a role in opiate reward. It is noteworthy that the VTA sends projections to the laterodorsal tegmental nucleus in addition to the adjacent TPP, though Semba and Fibiger (1992) asserted that "[retrograde] labeling in the ventral tegmental area was seen more consistently following TPP than LDT (laterodorsal tegmental) injections" (p. 393).

Given that glutamate and GABA receptors are involved in morphine reward in the TPP, the results obtained do not match any of the results predicted by the hypothetical anatomical models described in Figure 1. In the first category of scenarios in which the afferent, possibly VTA GABA neuron synapses onto a single output neuron, if glutamate is released from that neuron, a GABA antagonist should not block morphine reward (scenarios 1 & 2, Figure 1b). Alternatively, if GABA is released from the afferent neuron, the GABA agonist administered to the TPP should be rewarding (scenario 1, Figure 1a). With the models involving two serially connected neurons (scenarios 2, 3, & 4, Figure 1a; scenarios 3, 4, & 5, Figure 1b), the results also fail to match the predictions (Figure 7a). In the model with a GABA neuron connected to an output neuron, if GABA is released from the afferent neuron (scenarios 2 & 3, Figure 1a), it inhibits the GABA neuron that in turn releases the downstream output neuron from tonic inhibition. According to this setup, anything that releases the output neuron from the tonic GABA inhibitory signal from the upstream neuron, such as a GABA receptor antagonist in the TPP, should give reward, which does not agree with the results obtained. If glutamate is released from the afferent neuron (scenarios 3 & 4, Figure 1b) then a GABA agonist should yield reward, which again does not agree with the results. In the setup in which glutamate is released onto a glutamate neuron, which in turn synapses onto an output neuron (scenario 5, Figure 1b), we never make the prediction that a GABA antagonist will block morphine reward. In fact no matter where the GABA receptor is located, a GABA antagonist should yield reward by releasing either the upstream or downstream neuron from tonic GABA inhibition. Finally, if GABA was released, possibly from the VTA, onto a glutamate neuron in the TPP (scenario 4, Figure 1a), a GABA agonist should produce reward and a glutamate agonist should not. Neither prediction matches the results.

Although these are simple anatomical models, adding more neurons and receptors (either inhibitory receptors such as GABA or excitatory receptors such as glutamate) to the chain in a serial manner also fails to make predictions that match the results. We therefore must reject our original hypothesis of TPP anatomy with a serial or converging pathway underlying morphine reward.

Because blockade of mACh and GABA-A receptors failed to block the systemic morphine place preference, we can conclude that these receptors in the TPP are unlikely to be involved in systemic morphine reward.

Alternative Hypotheses

One possibility for an alternative hypothesis is that the VTA GABA cell synapses onto two different cells in the TPP, each with their own separate outputs. Indeed, only two separate and parallel reward pathways through the TPP can explain the current results. In these models, the VTA GABA cell is still presumed to release only one neurotransmitter type, either GABA or glutamate, and one simple model is proposed for each neurotransmitter (scenarios i & ii, Figure 7b). In these schemes, both pathways must be activated to produce reward. In the first, (scenario i, Figure 7b) in which GABA is released from the VTA cell onto two different GABA cells in the TPP, one pathway has three cells connected in series and the other pathway has two cells connected in series. The output of the three cell pathway is a reduction in neurotransmitter release from the output cell whereas the output of the two cell pathway is an increase in neurotransmitter output from the output cell. Together, the activity of these two output cells make up the reward signal. The result that GABA alone does not produce

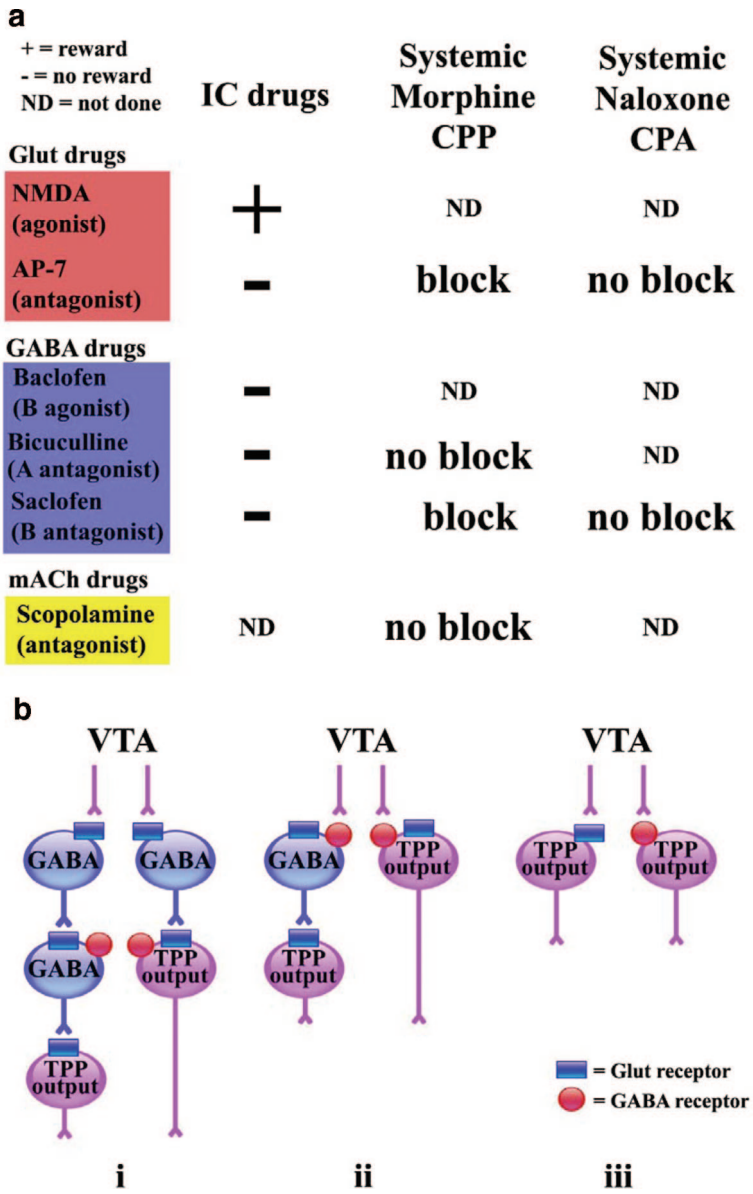

Figure 7. Summary of experimental results: The left column lists the intra-TPP drugs used whereas the top row indicates the experiments in which their effect was assessed. The first experiments (intracranial drugs), testing the motivational effect of the drugs on their own, showed that the only drug that had a rewarding effect was intra-TPP NMDA. The systemic morphine place preference was blocked by AP-7 and saclofen, but not by bicuculline or scopolamine. The systemic naloxone place aversion was not blocked by AP-7 or saclofen. ND indicates that the experiment was not performed. *Figure 7b. Alternative anatomical scenarios in the TPP*: (i.) GABA is released from VTA cells projecting to the TPP onto two separate GABAergic cells. One GABA cell then projects to an output neuron that has both a GABA and glutamate receptor and released GABA there. The other GABA cell projects to a second GABA cell in the TPP that has a glutamate receptor. This cell then projects to an output cell lacking a glutamate receptor and releases GABA there. (ii.) Glutamate is released from GABA cells of the VTA onto two separate cells in the TPP. One cell is an output neuron that bears both a glutamate and a GABA receptor. The other cell is a GABA cell that then projects to an output cell bearing only a GABA receptor and releases GABA there. In the above two cases, both pathways must be stimulated to produce reward. (iii.) The GABA projection from the VTA projects to two separate output cells in the TPP, one with only a GABA receptor and one with only a glutamate receptor. Each cell is stimulated only by its respective neurotransmitter type. With sufficient stimulation, only the glutamate receptor-bearing cell is needed to elicit reward, but in the case of systemic morphine reward, both output cells in the TPP are stimulated to produce reward. All of the above hypotheses make predictions about the motivational effects of GABA and glutamate agonists and antagonists that match the results obtained.

reward may be explained by the fact that although the output in the three cell pathway would agree with application of GABA to the TPP, in the two cell pathway GABA applied to the TPP would inhibit neurotransmitter release from the output cell. These results fail to match the reward output signal predicted in this setup. Here, the location of the NMDA receptor is vitally important to allow a proper rewarding signal when NMDA is applied to the TPP; an inhibition of the first (three neuron pathway) output cell and excitation of the second (two neuron pathway) output cell.

A simpler and arguably more plausible hypothetical model using two separate parallel pathways involves the release of glutamate from the VTA GABA cell (scenario ii, Figure 7b). In this case, one branch from the afferent connection synapses onto a GABA cell in the TPP, which in turn synapses onto an output cell, and another afferent branch synapses onto a single output cell. The output of the two-cell pathway is a reduction in neurotransmitter release whereas the output of the single-cell pathway is an increase in neurotransmitter release. This model successfully explains why the GABA-B agonist and antagonist have no motivational effects on their own, but the antagonist still blocks systemic morphine reward.

A third hypothesis involves the release of different transmitters from afferent, possibly VTA, cells onto separate pathways in the TPP (scenario iii, Figure 7b). The system involves two separate and parallel TPP output cells, one responding to a GABA input, possibly from the VTA, and one responding to a glutamate input (again, possibly from the VTA). An important concession that must be made for this hypothesis to match the experimental results is that the cell with the glutamate receptor, if stimulated to a sufficient degree, must be capable of producing reward on its own because the administration of NMDA in the TPP produces reward. However, in the case of systemic morphine reward, both pathways must be active (because GABA-B and NMDA antagonists alone block morphine reward), with neither the glutamate nor the GABA signal being strong enough on its own to elicit reward. An interesting test of the latter model would involve titrating intra-TPP NMDA reward to just below threshold, then adding a GABA-B agonist to see if reward can be elicited. If so, this would lend support to scenario iii in Figure 7b, and the idea that morphine causes a weak activation of both the glutamate pathway and the GABA pathway to produce its rewarding effects.

Some studies have pointed to the TPP as having a role in learning or response selection rather than mediating the motivational rewarding effects of certain stimuli (Alderson & Winn, 2005; Steiniger-Brach & Kretschmer, 2005). In this way, TPP lesions may produce response selection deficits whereby animals can still experience reward, but cannot respond appropriately (Inglis, Olmstead, & Robbins, 2000; Steiniger-Brach & Kretschmer, 2005). Alderson and Winn, citing self-administration studies (Alderson, Latimer, Blaha, Phillips, & Winn, 2004; Olmstead et al., 1998) suggested that the reinforcement process—not reward itself—had been impaired. One possibility in the present study is that the antagonists used are indeed producing deficits in response selection or learning or even in the ability of the animal to sense the cues relevant to the place conditioning experiment.

However, if the intra-TPP antagonists were causing the deficits mentioned above, animals would similarly be unable to make the appropriate response to an aversive stimulus (avoidance of the naloxone-paired compartment) after conditioning with naloxone. The naloxone experiments demonstrate that under the influence of the intra-TPP antagonists, they still are able to move to one side of the apparatus. This choice on the place conditioning test is therefore not specifically blocked by TPP manipulations, nor is the ability to sense the relevant cues.

The two separate parallel pathways through the TPP implied by the results of the current study lead to questions regarding the exact output of the TPP and its projections with respect to morphine reward. Despite the prevalence of ascending cholinergic projections from the TPP, these are not likely candidates for reward output considering evidence that cholinergic cell loss in the TPP does not coincide with a block of morphine reward (Olmstead & Franklin, 1993). Furthermore, though there are ascending reciprocal projections to midbrain DA neurons (Mena-Segovia et al., 2004) and even though lesions of the TPP diminish the ability of morphine to elicit DA efflux in the striatum (Miller et al., 2002), these projections are unlikely to be involved in reward because disruption of midbrain DA transmission has no effect on morphine reward in the drug naive condition (Hnasko, Sotak, & Palmiter, 2005; Mackey & van der Kooy, 1985; Olmstead & Franklin, 1997). This leaves the possibility of descending (possibly glutamatergic) projections as the ones that importantly mediate morphine reward (Miller et al., 2002), though other ascending projections to non-DA regions are by no means ruled out. The present data are more consistent with two parallel glutamate and/or GABA reward output pathways from the TPP mediating morphine reward.

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