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BLOCKADE OF VENTRAL PALLIDAL OPIOID RECEPTORS INDUCES A CONDITIONED PLACE AVERSION AND ATTENUATES ACQUISITION OF COCAINE PLACE PREFERENCE IN THE RAT

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Abstract—Peripheral administration of naloxone is known to produce a conditioned place aversion and to block cocaine-induced conditioned place preference. The ventral pallidum receives a dense enkephalinergic projection from the nucleus accumbens and is implicated as a locus mediating the rewarding and reinforcing effects of psychostimulant and opiate drugs. We sought to provide evidence for the involvement of pallidal opioid receptors in modulating affective state using the place-conditioning paradigm. Microinjection of naloxone (0.01–10 μg) into the ventral pallidum once a day for 3 days dose-dependently produced a conditioned place aversion when tested in the drug-free state 24 h after the last naloxone injection. This effect was reproduced using the μ-opioid receptor selective agonist D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (CTOP, 1 μg). Locomotor activity was reduced following injection of the highest dose of naloxone (10 μg) but elevated following CTOP (1 μg). Daily injection of cocaine (10 mg/kg) for 3 days produced a conditioned place preference 24 h later. This effect of cocaine was attenuated by concomitant intra-ventral pallidal injection of naloxone at a dose (0.01 μg) that had no significant aversive property when injected alone. In contrast, the locomotor activation induced by peripheral cocaine injection was unaffected by naloxone injection into the ventral pallidum.

The data implicate endogenous opioid peptide systems within the ventral pallidum as regulators of hedonic status.

Key words: CTOP, naloxone, opiate, pallidum, psychostimulant.

Exogenous opiate administration produces reward and promotes reinforcement of drug-seeking behavior (van Ree et al., 1999; Di Chiara and North, 1992). Furthermore, activation of endogenous opioid peptide release may be an important component in the rewarding and reinforcing effects of other abused substances, such as alcohol (Altshuler et al., 1980; Hall et al., 2001; Herz, 1997, 1998; Kornet et al., 1991) and psychostimulants (Kosten et al., 1992; Kim et al., 1997; Kuzmin et al., 1997; Mello and Negus, 1996; Suzuki et al., 1992, 1994; Gerrits et al., 1995; Mitchem et al., 1999; Ramsey and van Ree, 1991). Conversely, disruption of tonic activity within endogenous opioid circuitry by administration of naloxone or naltrexone has been reported to induce dysphoria in humans (Hollister et al., 1981; Grevert and Goldstein, 1977a) and produces powerful conditioned aversive responses in animals (Grevert and Goldstein, 1977b; Mucha and Iversen, 1984; Mucha et al., 1982, 1985; Mucha and Herz, 1985; Mucha and Walker, 1987; Iwamoto, 1985; Parker and Rennie, 1992). Consequently, it has been proposed that endogenous opioid systems are important factors in the maintenance of "hedonic homeostasis" (Koob and Le Moal, 1997).

The aversive effect of naloxone is centrally mediated since methylated analogues of this drug, which do not readily penetrate the blood–brain barrier, do not produce conditioned place aversion (CPA) when administered peripherally (Hand et al., 1988; Kuzmin et al., 1997), whereas i.c.v. injections of naloxone are effective in this regard (Bals-Kubik et al., 1989). However, the site(s) of action within the brain for the aversive effect of naloxone has received little attention. The mesolimbic dopamine pathway was the target of initial study given the proposed importance of this system in mediating the rewarding effects of opiate drugs. Thus, injection of naloxone into the ventral tegmental area (VTA) or nucleus accumbens (but not the medial prefrontal cortex or caudate/putamen) were shown to induce CPA (Shippenberg and Bals-Kubik, 1995). However, in the same study, intra-nucleus accumbens 6-hydroxydopamine-induced depletion of mesolimbic dopamine failed to block the ability of naloxone to induce CPA after injection into this region. Therefore, just as there is evidence of dopamine-independent sources of opiate reward (Pettit et al., 1984; Dworkin et al., 1988a), there seems to exist a mechanism, perhaps downstream of dopamine terminals in the nucleus accumbens, which is capable of mediating an aversive action of naloxone independent of mesolimbic dopamine activity. Similarly, the site of action for opioid antagonist's inhibitory action on the rewarding or reinforcing effects of alcohol and psychostimulants has received little scrutiny. Circumstantial evidence for an interaction with dopamine systems exists by virtue of the fact that local injection of naltrexone into the VTA (but not the nucleus accumbens, caudate putamen, amygdala or prefrontal cortex) attenuates cocaine self-administration (Ramsey et al., 1999).

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Abbreviations: ANOVA, analysis of variance; AP, anterior–posterior; CPA, conditioned place aversion; CPP, conditioned place preference; CTOP, D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2; DDI, double deionized; ML, medial–lateral; V, dorso–ventral; VP, ventral pallidum; VTA, ventral tegmental area,

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The ventral pallidum (VP) is a major efferent target of nucleus accumbens enkephalin-containing cells (Chrobak and Napier 1993; Napier et al., 1983; Zahm et al., 1985), is rich in opioid receptors (Olive et al., 1997; Mitrovic and Napier, 1995; Mansour et al., 1988, 1995a,b) and is increasingly acknowledged as a key region in reward/reinforcement circuitry (Wilson and Rolls, 1990; Johnson et al., 1993; McAlonan et al., 1993; Johnson and Stellar, 1994a,b; Panagis et al., 1995, 1998; Hubner and Koob, 1990; Robledo and Koob, 1993; Gong et al., 1996, 1997). Furthermore, activation of opioid receptors in this region has been shown to modulate the activity of VP neurons (Napier and Mitrovic, 1999; Mitrovic and Napier, 1995).

For these reasons, we considered the VP as a possible target for naloxone's aversive effect and tested the ability of this general opioid antagonist to induce CPA after local administration in the VP of rats. In a separate experiment, the role of μ-opioid receptor blockade in mediating aversion was investigated by administering the μ receptor selective antagonist, D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-Nh_2 (CTOP), into the VP. Subsequently, we examined the importance of VP endogenous opioid peptides in mediating cocaine reward by testing the ability of a non-aversive dose of intra-VP naloxone to block acquisition of cocaine conditioned place preference (CPP).

EXPERIMENTAL PROCEDURES

Subjects

Adult male Sprague–Dawley rats obtained from Harlan (Indianapolis, IN, USA) (175–225 g) were handled and weighed daily for 5 days prior to the start of the experiment. Subjects were housed in groups of two or three in transparent (9×19×8 cm) plastic cages and received food and standard laboratory rat chow *ad libitum*. The colony was maintained on a 12-h light/dark cycle, lights on 0800–2000 h at an ambient temperature of 68–70 °C. All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and all efforts were made to minimize the number of animals used and their suffering.

Surgery

Guide cannulae were implanted employing standard stereotaxic procedures. Subjects were anesthetized with 2–3% halothane in O_2/N_2O (1:1) reduced to 1% after induction. Twenty-four-gauge guide cannulae (Plastics One, Roanoke, VA, USA), were positioned unilaterally 2.2 mm above the VP (anterior–posterior (AP) −0.2 mm, medial–lateral (ML) +2.0 mm, dorso–ventral (V) 5.0 mm, according to the atlas of Paxinos and Watson, 1986). A stylet was placed in the guide and removed to allow for injections. Subjects were allowed 3–5 days to recover before behavioral tests began. During conditioning sessions drug or vehicle was injected in a volume of 0.5 μl through the guide using a 31-gauge injector needle (Plastics One) terminating in the V.P. at a depth of 7.7 mm below the skull surface.

Apparatus

The conditioning apparatus allowed for automated recording of subject location (time spent in each chamber) and locomotor activity (Coulbourn Instruments, Allentown, PA, USA). The square arena of the apparatus was divided into three chambers by a Plexiglas partition. Two chambers, identical in size, shape (trapezoid) and texture (smooth) were used as the conditioning chambers. One of these chambers was decorated on all four walls and floor with black and white checkered (2-cm squares) contact paper and contained almond extract (McCormick and Co., Hunt Valley, MD, USA) as an olfactory cue (200 μl on a strip of filter paper hung from the top corner of the chamber). The other chamber was decorated on all four walls and floor with black and white cow-print contact paper (RubberMaid, Wholly Cow pattern Rubbermaid, Wooster, OH, USA) and was accentuated with lemon extract (McCormick and Co.) presented in the same fashion. The visual cues offered approximately equal amounts of white and black. A smaller triangular-shaped chamber decorated with gray contact paper on all three walls and floor was designated the "start" chamber. This neutral chamber remained odorless. Two removable guillotine doors allowed access from the start chamber to each of the conditioning compartments. Animals were placed directly into this start chamber during habituation and test sessions.

Habituation

On day 1, subjects were placed in the "start" chamber and permitted free access to the entire apparatus for 15 min. The time spent in each of the chambers was recorded to measure any initial bias.

Conditioning

Experiments 1 and 2. On days 2–4, in the morning, animals received an intra-VP injection of filter-sterilized double-deionized (DDI) water (referred to below as 'placebo' treatment) and were confined to the 'placebo-paired' chamber for 30 min and subsequently returned to the home cage after the stylet was replaced. Four hours later, subjects received a unilateral intra-VP injection of naloxone (0.01, 0.1, 1 or 10 μg), CTOP (1 μg) or DDI water, and were confined to the 'drug-paired' chamber for 30 min. The drug-paired chamber was randomized across subjects. Locomotor activity was also recorded during each of the six conditioning sessions.

Experiment 3. On days 2–4, in the morning, animals received a unilateral intra-VP injection of DDI water followed immediately by an i.p. injection of saline (referred to below as 'placebo' treatment) and were confined to the 'placebo-paired' chamber for 30 min. Five hours later, subjects received one of the following drug combinations: DDI water (intra-VP) plus cocaine (10 mg/kg, i.p.); naloxone (0.01 μg, intra-VP) plus cocaine (10 mg/kg, i.p.); DDI water (intra-VP) plus saline (i.p.) and were confined to the 'drug-paired' chamber for 30 min. The drug-paired chamber was randomized across subjects.

Test

On day 5, 24 h after the last drug treatment, subjects, in a drug-free state, were allowed to freely explore the apparatus with the doors removed for 15 min. The time spent in each chamber was recorded.

Histology

At the conclusion of the experiment, rats were deeply anesthetized with pentobarbitol (100 mg/kg, i.p.) and trans-cardially perfused with 0.9% saline and 10% formalin saline. Brains were removed and postfixed for at least 1 day in formalin. Brains were cryosectioned into 50 μm slices, mounted onto slides, and stained with Cresyl Violet. Correct placement of injection sites (Paxinos and Watson, 1986) was confirmed. Animals with misdirected injection sites were either analyzed separately (experiment 1) or dropped from the statistical analysis (experiments 2 and 3).

Drugs

Cocaine HCl (Sigma) was dissolved in 0.9% saline and naloxone HCl (generously provided by NIDA) or CTOP (Bachem, Torrance, CA, USA) was dissolved in filter-sterilized (Sterile Acrodisc, 0.2 μm, Gelman Sciences) DDI water.

Statistical analysis

The time spent in the drug-paired chamber on the test day was averaged across subjects in each group and analyzed by one-way analysis of variance (ANOVA) followed by Fisher's protected LSD post hoc tests (experiments 1 and 3) or by Student's *t*-test (experiment 2). $P \leq 0.05$, one-tailed, was considered statistically significant. Locomotor activity summated across the three drug-conditioning sessions was similarly analyzed.

RESULTS

Experiment 1

Analysis of habituation data showed no preconditioning bias for either conditioning chamber across all animals ($F_{1,100}=0.642$; $P>0.05$) (data not shown). Moreover, after random allocation to drug-treatment groups, time spent in the future drug-paired chamber during the habituation period was balanced across the five treatment groups (vehicle, naloxone 0.01, 0.1,1, 10 μg) ($F_{4,46}=0.279$; $P>0.05$; data not shown).

Intra-VP naloxone produced a dose-dependent aversion (Fig. 1A). Statistical analysis of time spent in the drug-paired chamber on the test day revealed a significant effect of drug treatment ($F_{4,36}=6.028$; $P<0.05$). The post hoc test showed that naloxone produced a significant place aversion in rats treated with 0.1, 1 or 10 μg of naloxone ($P<0.05$), but did not have any effect at a dose of 0.01 μg ($P>0.05$). This effect was also site-specific, as 1 or 10 μg of naloxone injected into surrounding regions (mainly the striatum) failed to decrease time spent in the drug-paired chamber compared with vehicle ($F_{2,19}=0.217$; $P>0.05$) (Fig. 1A). A schematic representation of naloxone injection sites is given in Fig. 4A.

Locomotor activity, expressed as the total distance traveled during the three drug-conditioning sessions, was also affected by naloxone administration ($F_{4,29}=5.894$, $P<0.05$) (Fig. 1B). However, only the highest dose tested (10 μg) produced a significant decrease in locomotion compared with vehicle (post hoc analysis, $P<0.05$). Misdirected injection of the highest doses of naloxone (1 and 10 μg) into surrounding regions had no effect on locomotor activity (Fig. 1B).

Experiment 2

Analysis of habituation data using a one-factor ANOVA (chamber) showed no preconditioning bias for either conditioning chamber across all animals ($T_{1,18}=0.362$; $P>0.05$) (data not shown). Moreover, after random allocation to drug treatment groups, time spent in the future drug-paired chamber during the habituation period was balanced across the two treatment groups (vehicle, CTOP 10 μg) ($T_{1,8}=0.401$; $P>0.05$) (data not shown).

On the test day, rats treated with CTOP spent significantly less time in the drug-paired chamber compared with the vehicle group ($T_{1,8}=1.953$; $P<0.05$) (Fig. 2A). Placement of CTOP injections is shown in Fig. 4B. In contrast to animals treated with naloxone (experiment 1), animals treated with CTOP displayed a significant increase in locomotor activity ($F_{1,8}1.894$; $P<0.05$) (Fig. 2B).

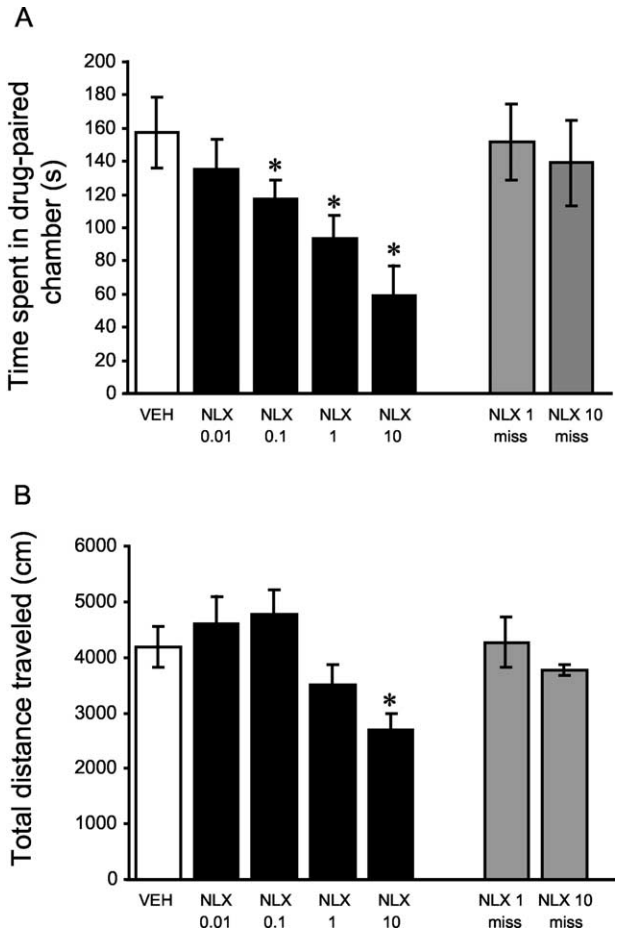

Fig. 1. (A) Naloxone (NLX 0.1, 1.0, 10.0 μg, intra-VP) produced a significant CPA. Misdirected microinjections into surrounding tissue had no effect. Bars represent mean±S.E.M. time spent in drug-paired chamber. * $P<0.05$ compared with vehicle group. (B) The highest does of naloxone (10 μg, intra-VP) produced a significant decrease in horizontal locomotor activity. Bars represent mean±S.E.M. total distance traveled over the three drug-conditioning sessions. * $P<0.05$ compared with vehicle group.

Experiment 3

Analysis of habituation data showed no preconditioning bias for either conditioning chamber across all animals ($F_{1,48}=0.703$; $P>0.05$) (data not shown). Moreover, after random allocation to drug-treatment groups, time spent in the future drug-paired chamber during the habituation period was balanced across the three treatment groups (vehicle, cocaine, cocaine plus naloxone) ($F_{2,22}=1.555$; $P>0.05$) (data not shown).

Cocaine conditioning produced a significant place preference, the acquisition of which was attenuated by intra-VP pretreatment with naloxone (Fig. 3A). Statistical analysis of time spent in the drug-paired chamber on the

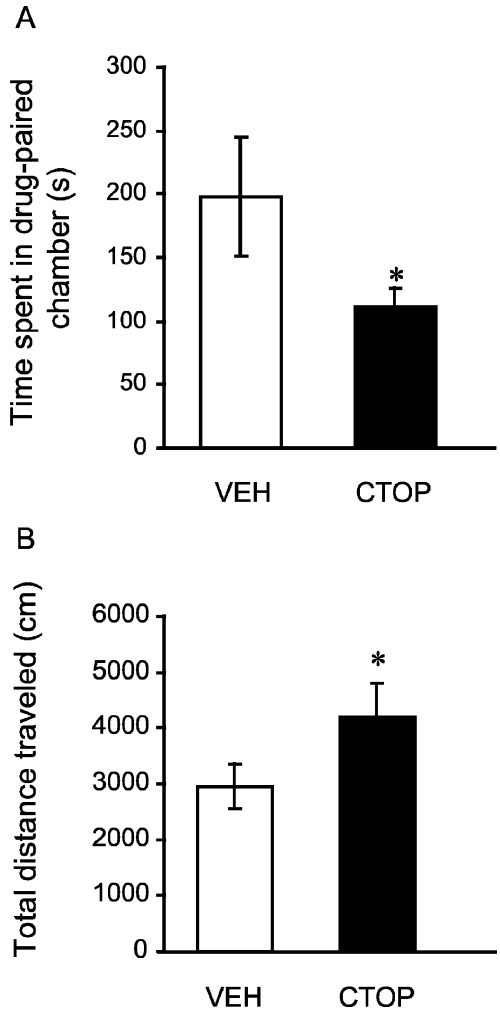

Fig. 2. (A) CTOP (1 μg, intra-VP) produced a significant CPA. Bars represent mean±S.E.M. time spent in drug-paired chamber. * $P<0.05$ compared with vehicle group. (B) CTOP (1 μg, intra-VP) produced a significant increase in horizontal locomotor activity. Bars represent mean±S.E.M. total distance traveled over the three drug-conditioning sessions. * $P<0.05$ compared with vehicle group.

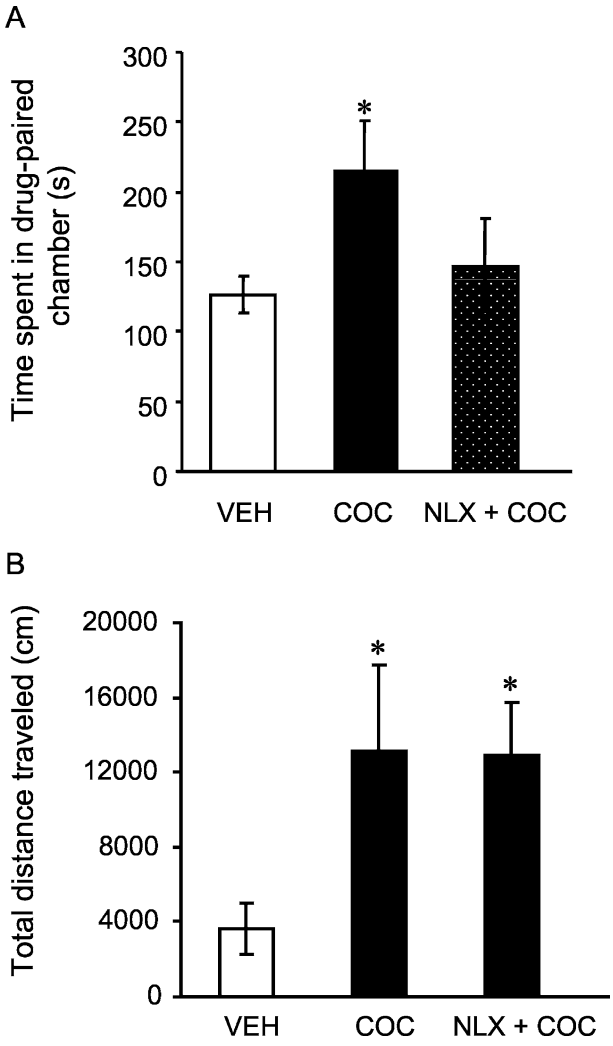

Fig. 3. (A) Cocaine (10 mg/kg, i.p.) produced a significant CPP compared with the vehicle group and this effect was blocked by pretreatment with naloxone (0.01 μg, intra-VP). Bars represent mean±S.E.M. time spent in drug-paired chamber. * $P<0.05$ compared with vehicle group. (B) Cocaine (10 mg/kg, 1.p.) produced a significant increase in horizontal locomotor activity; and this effect was not blocked by pretreatment with NLX (0.01 μg, intra-VP). Bars represent mean±S.E.M. total distance traveled over the three drug-conditioning sessions. * $P<0.05$ compared with vehicle group.

test day revealed a significant main effect of drug treatment ($F_{2,20}=3.144$; $P<0.05$) and post hoc analysis showed that the cocaine alone group spent significantly more time in the drug-paired chamber than both the vehicle and the cocaine plus naloxone groups ($P<0.05$). Sites of these naloxone injections are given in Fig. 4C.

In contrast, intra-VP naloxone failed to block the locomotor stimulatory effect of cocaine (Fig. 3B). Statistical analysis revealed a significant main effect of drug treatment ($F_{2,9}=12.923$; $P<0.05$) and post hoc tests showed that both the cocaine alone and cocaine plus naloxone groups exhibited significantly increased locomotor behavior compared with vehicle-treated animals ($P<0.05$).

DISCUSSION

We have demonstrated that microinjection of the general opioid antagonist, naloxone, into the VP dose-dependently induces a CPA in rats. This effect appears to involve the μ-opioid receptor, as the selective μ antagonist, CTOP, administered locally into this region, was similarly able to produce a CPA. Furthermore, pretreatment with ventral pallidal injection of naloxone, at a dose that alone did not produce aversion, blocked the acquisition of cocaine CPP, without affecting cocaine-induced hyperactivity. Overall, the data implicate the VP as an important locus for maintaining hedonic status.

The VP is anatomically well situated within the limbic system to fulfill such a role. It receives major inputs from the nucleus accumbens, previously described as containing enkephalins (Napier et al., 1983; Zahm et al., 1985), substance P (Napier et al., 1995), dynorphin (Haber and Watson, 1985) and GABA (Zahm et al., 1985). The VP also

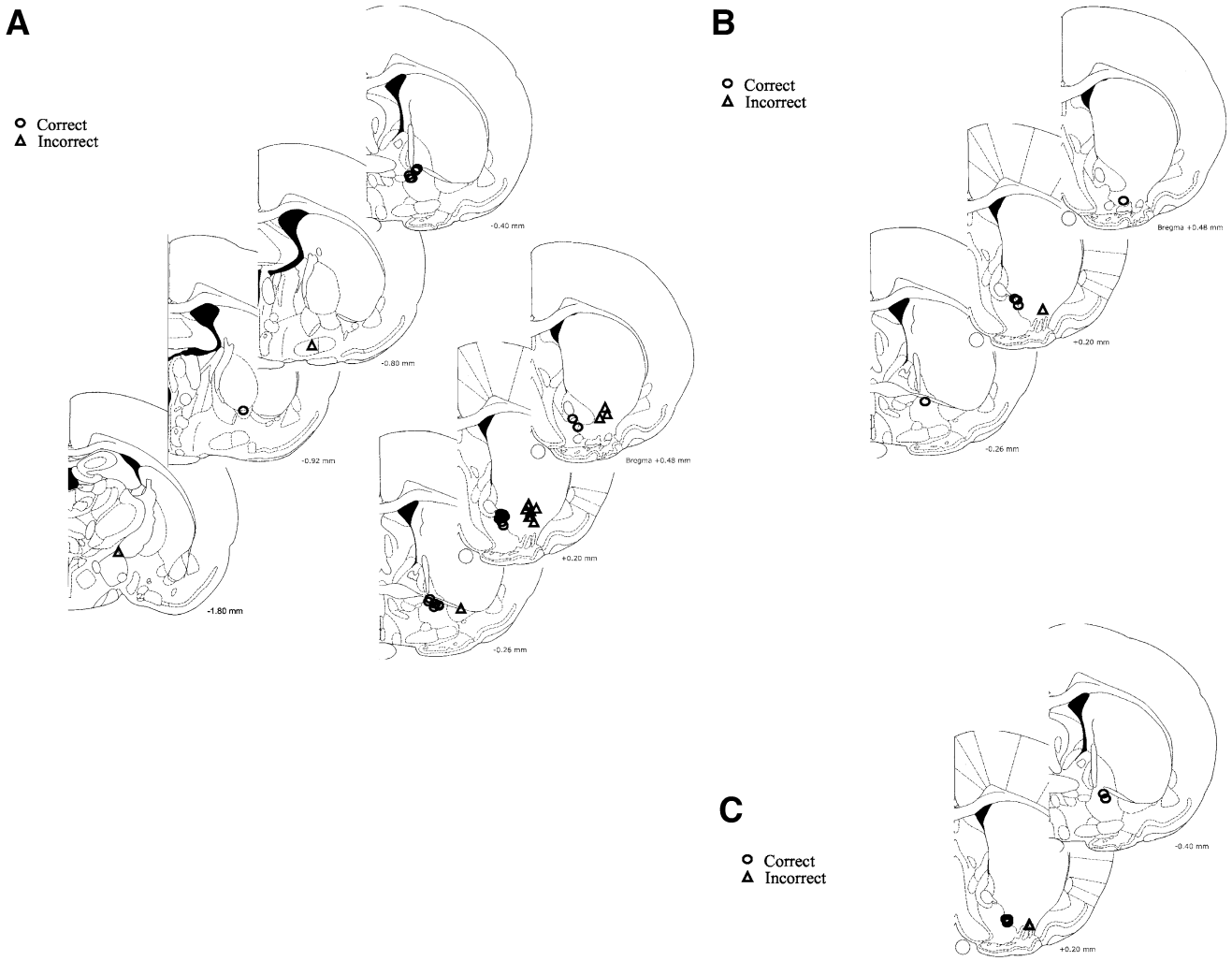

Fig. 4. (A) Schematic representation of naloxone injection sites from experiment 1. 10 μg group (n=8 hit, n=9 miss); 1 μg group (n=9 hit, n=5 miss); 0.1 μg group (n=8 hit); 0.01 μg (n=7 hit). (B) Placement of CTOP (1 μg) injections from experiment 2 (n=5 hit, n=1 miss). (C) Sites of NLX (0.01 μg) injections used to block cocaine CPP from experiment 3 (n=8 hit, n=2 miss). Open circles indicate correct placements, while open triangles represent incorrect placements in all cases.

receives dopaminergic projections from the VTA and substantia nigra (Napier and Potter, 1989; Klitenick et al., 1992) in addition to glutamatergic inputs from the frontal cortex (Toan and Schultz, 1985), amygdala (Fuller et al., 1987) and subthalamic nucleus (Robledo and Feger, 1990; Brotchie and Crossman, 1989). VP neurons are mainly cholinergic or GABAergic (Zaborszky et al., 1999) and target regions include the nucleus accumbens, VTA, prefrontal cortex, amygdala, lateral hypothalamus, mediodorsal thalamic nucleus, subthalamic nucleus and substantia nigra (Carlsen et al., 1985; Zaborszky et al., 1999; Groenewegen et al., 1993; Haber et al., 1985; Young et al., 1984). Thus, the VP shares extensive reciprocal connections with many structures known to be involved in regulating reward, including the nucleus accumbens and VTA, which, like the VP, have also been shown to mediate opioid antagonist-induced conditioned aversion (Shippenberg and Bals-Kubik, 1995). Our finding that the striatum was a non-responsive site to misguided naloxone injections is consistent with the report from Shippenberg and Bals-Kubik (1995).

As noted above, the VP receives a rich enkephalinergic innervation from the nucleus accumbens (Zahm et al., 1985). Met- and Leu-enkephalin are agonists at both μ- and δ-opioid receptors and both of these receptors are abundant in the VP of the rat and several other species (Pilapil et al., 1987; Lahti et al., 1989; Moskowitz and Goodman, 1984; Mansour et al., 1988, 1995a,b; Olive et al., 1997). The activity of VP neurons has been shown to be regulated by opioid receptors, both directly via activation of opioid receptors present on VP neurons, and indirectly through pre-synaptic modulation of afferents (Napier and Mitrovic, 1999). About 30% of neurons in the VP are regulated by all three major types of opioid receptor—μ, δ and κ—whereas about 25% respond only to μ-receptor activation, and an additional 30% are not regulated by any opioid ligands (Mitrovic and Napier, 1995). VP neurons that respond to opioid agonists do so with either suppres-

sion or an increase of firing (Mitrovic and Napier, 1995). The increased activity is most likely a result of presynaptic inhibition of inhibitory inputs (Napier and Mitrovic, 1999). Whether the behavioral effects observed in the current study are a result of pre- or post-synaptic activation is currently unknown. Our observation that CTOP was effective in producing a CPA after intra-VP injection implicates blockade of the μ receptor in the VP as a mediator of aversion, and we have previously shown this receptor to be located both pre- and post-synaptically in the VP (Olive et al., 1997). CTOP is also effective after i.c.v. (Bals-Kubik et al., 1989), intra-VTA and intra-nucleus accumbens injection (Shippenberg and Bals-Kubik, 1995). The conclusion that blockade of μ-opioid receptors is important in this regard is supported by the total absence of a peripherally administered naloxone-induced CPA in mice deficient in the μ-opioid receptor (Skoubis et al., 2001). Furthermore, δ-selective antagonists are ineffective in producing CPA in opiate-naive rats and mice (Shippenberg et al., 1987; Bals-Kubik et al., 1989; Menkens et al., 1992; de Vries et al., 1995; Skoubis et al., 2001).

The simplest explanation for naloxone's aversive action in the VP is that it is blocking the effect of a tonically released opioid peptide ligand involved in mediating a positive affective state. Previous study pointed to the β-endorphins as mediating such a tonic influence since lesions of the arcuate nucleus of the hypothalamus, the major source of β-endorphin neurons in the brain, apparently attenuate peripherally administered naloxone-induced aversion (Mucha et al., 1985). However, the rich enkephalinergic innervation of the VP, alluded to above, would implicate these peptides. Indeed, recent data from our laboratory (to be presented elsewhere), using opioid peptide precursor-deficient mice, strongly implicate enkephalins rather than endorphins as the endogenous ligands mediating such a basal "hedonic tone."

Although we showed that our effective injection sites were located in the VP, we cannot discount the possibility that the behavioral effect was caused by diffusion of naloxone to neighboring regions such as the diagonal band, preoptic area, or olfactory tubercle. Also, since naloxone was administered as a hydrochloride salt, there was a concern that the observed effects of naloxone injections were nonspecific effects of low pH. We therefore conducted an additional control experiment comparing intra-VP injection of HCl solution (pH=5.0 to match that of the highest dose of naloxone used previously) with injection of DDI water. This HCl injection was ineffective in establishing a CPA compared with the control group ($F_{1,12}=0.476$; $P>0.05$, $n=8$; data not shown).

Previous studies have reported that systemically administered naloxone or naltrexone block the acquisition of cocaine CPP (Kim et al., 1997; Kuzmin et al., 1997; Suzuki et al., 1992; Gerrits et al., 1995). In the present study, cocaine produced a significant CPP that while somewhat small in absolute terms (on average 90 s longer in the "drug-paired" chamber out of the total 900-s recording period), nevertheless represents a 70% increase over time spent in the vehicle-paired chamber due to the fact that animals spent most of their time in the neutral chamber. Pre-injection of only 0.01 μg of naloxone into the VP, a dose that had no significant aversive effect when administered alone, significantly attenuated this cocaine CPP. To our knowledge, these are the first data to directly implicate the VP as a possible site of action for naloxone in blocking cocaine CPP. Other lines of evidence have, however, previously pointed to the general importance of this structure for both psychostimulant and opiate reward. For instance, excitotoxic lesions of the VP produce significant decreases in both cocaine and opiate self-administration (Dworkin et al., 1988b; Hubner and Koob, 1990; Robledo and Koob, 1993). The VP may in fact be an important site for cocaine's direct action on dopamine systems. As noted above, dopaminergic terminals are present in the VP and local injection of cocaine into this structure induces a CPP (Gong et al., 1996), an effect reversed by 6-hydroxydopamine lesions of the VP (Gong et al., 1997). Similarly to the case with naloxone's aversive effects at higher doses, it is unclear at this time if naloxone is blocking cocaine CPP via pre- or post-synaptic mechanisms within the VP. Furthermore, although we were careful to use a dose of naloxone that had no significant aversive effect alone, we cannot completely rule out the possibility that the observed attenuation of cocaine CPP results from a balance of naloxone aversion and cocaine reward rather than interfering with cocaine reward mechanisms per se.

Given the potent effect of higher doses of naloxone alone when injected into the VP, this structure may well represent a focal point for the regulation of "hedonic homeostasis" (Koob and Le Moal, 1997) and may also be important in mediating the aversive stimulus effects of opiate withdrawal. It is important to note that our study employed opiate-naïve animals. Previous work has shown that, in morphine-dependent animals, much lower doses of naloxone (doses that produce no obvious signs of physical withdrawal) are capable of producing a CPA (Mucha, 1987; Schulteis et al., 1994) and that this aversive stimulus effect is very long lasting (Stinus et al., 2000). Site-specific injections of methylnaloxonium point to the nucleus accumbens and amygdala as potential mediators under these conditions (Stinus et al., 1990) but it would be interesting to examine the VP in such a model in future studies in light of the present data. Interestingly, in the morphine-dependent state, δ-receptor antagonists elicit a CPA (Funada et al., 1996) despite their apparent lack of effect in opiate-naïve animals as noted above.

Our observation that intra-VP naloxone, at the highest dose tested, reduced motor activity is consistent with the majority of reports of hyperlocomotion following injection of μ- or δ-agonists into this region (Austin and Kalivas, 1990; Hoffman et al., 1991; Napier, 1992; Johnson et al., 1993; Johnson and Stellar, 1994). However, the aversive effect of naloxone does not appear to be a simple by-product of this reduced activity since aversion was produced at doses that were not sufficient to induce hypolocomotion. Moreover, the aversive effect of CTOP was accompanied by an increase, rather than a decrease, in activity. Similarly, intra-VP naloxone blocked cocaine CPP without affecting

the hyperlocomotion produced by this psychostimulant. The reason for the apparently anomalous hyperactivity response to CTOP is unclear but may reflect heterogeneity in μ-receptor function within the VP. The μ agonist, DAMGO, has been shown to produce differential effects on motor behavior depending on the site of injection within this structure: DAMGO injected into the caudal VP increased motor behavior, whereas the same drug decreased motor activity when injected in the rostral VP (Johnson et al., 1993). Since our injections targeted the medial rostral region of the VP the hyperlocomotor response observed with CTOP may be considered consistent with the data of Johnson et al. (1993). It should also be noted that low doses of naloxone produced a non-significant trend toward hyperactivity and it is possible that the hypolocomotor effect of the high dose of naloxone involves blockade of δ as well as μ receptors, the effect of which may differ from μ-receptor blockade alone.

We conclude that the VP may be an important site mediating naloxone-induced CPA in rats, and that selective blockade of μ-opioid receptors within the VP is sufficient for the acquisition of opiate antagonist-induced CPA, consistent with previous data from μ-receptor knockout mice (Skoubis et al., 2001). We have also shown that opioid activity at the level of the VP is necessary for the acquisition of cocaine CPP. In light of recent data showing that opioid peptide release may be enhanced in this region immediately prior to cocaine self-administration (Gerrits et al., 1999), opioid peptides in the VP may be critical not only for "hedonic homeostasis" but also for drug anticipation states and, therefore, possibly relapse. Indeed VP injections of CTOP were recently shown to block the development of morphine locomotor sensitization (Johnson and Napier, 2000), a phenomenon hypothesized to reflect a key component of the addictive process (Robinson and Berridge, 2001).

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