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Intra-ventral tegmental area heroin-induced place preferences in rats are potentiated by peripherally administered alprazolam

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Abstract

The present experiment was designed to replicate and extend previous results of an opiate+benzodiazepine interaction in which peripherally administered alprazolam was observed to modulate behavior resulting from intravenous injections of heroin. As a first step in determining the role of central sites in this drug interaction, changes in drug reward (measured by conditioned place preference; CPP) were assessed in rats given systemic administration of alprazolam coupled with intracranially infused heroin (into the ventral tegmental area; VTA). Sprague–Dawley rats were implanted with guide cannula targeting the VTA, after which a heroin-induced CPP dose–response curve was determined (2.5–40 ng administered bilaterally in 0.5 μl/side). In other animals, intra-VTA heroin-induced place preferences were challenged with systemically applied alprazolam (0.125 mg/kg IP). The data confirm that rats dose-dependently develop reliable place preferences for a distinct environment paired with bilateral VTA-infusions of heroin. Additionally, when a non-rewarding dose of alprazolam was combined with a non-rewarding bilateral intra-VTA heroin dose (5 ng), a significant CPP was produced. These data extend earlier results by demonstrating that a systemically applied benzodiazepine can enhance the rewarding effects produced by central opiate administration. The results suggest that the VTA might be a site where this opiate+benzodiazepine interaction occurs.

Keywords: Heroin; Opiate; Alprazolam; Benzodiazepine; Place preference; Reward; Reinforcement; Ventral tegmental area; VTA; Drug interaction

Clinical research has suggested that human opiate users often self-administer benzodiazepines (BDZs) either prior to, or concurrently with, opiates (Stitzer et al., 1981; Preston et al., 1984; Weddington and Carney, 1987; Navaratnam and Foong, 1990a; Forsyth et al., 1993; Iguchi et al., 1993). The primary explanation for this co-administration is the reported potentiation of the opiate reward experience produced by the BDZ pretreatment (Stitzer et al., 1981; Navaratnam and Foong, 1990a; Gelkopf et al., 1999). Consistent with this view is a report on a small sample of five adult male patients maintained in a methadone maintenance program. These opiate users described the subjective effects of concurrently administered opiate+BDZ (Op–BDZ) as more pleasurable than either one of the drugs taken alone (Preston et al., 1984). Such an action might account for the high incidence of co-administration of the two drugs by way of a financial savings since BDZ pretreatment may permit the user to experience a comparable euphoric effect with a smaller dose of opiate and hence extend the number of doses that a given quantity of opiate can provide (Navaratnam and Foong, 1990a).

The prevalence of Op–BDZ co-abuse is widespread with clinical reports from Europe, Asia, the Middle East, Australia and the United States (Segura et al., 2001; Gelkopf et al., 1999; Darke et al., 1995; Iguchi et al., 1993; Navaratnam and Foong, 1990a). For example, 99% of patients that had entered an opiate detoxification center in Malaysia reported that they had co-abused opiates and BDZs within the 24-h period prior to detoxification (Navaratnam and Foong, 1990b). Another recent clinical report focusing on BDZ use in methadone treatment centers located in Spain, established that Op–BDZ co-abuse was occurring in 48% of their patient population (Segura et al., 2001). A similar analysis provided by an Israeli study found that 66.6% of the opiate-using patient population abused BDZs in the 12-month period before beginning methadone mainte-

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nance, with 41.4% of those patients reporting use of BDZs to enhance the effect of the opiate (Gelkopf et al., 1999).

In order to systematically study the mechanisms by which BDZs might potentiate opiate reward, Walker and Ettenberg (2001) utilized the conditioned place preference test in laboratory animals to measure the rewarding effects of Op–BDZ interactions. The results of the study indicated that a single dose of the BDZ, alprazolam (0.125 mg/kg), potentiated the rewarding properties of a low heroin dose (0.025 mg/kg IV) that was itself non-rewarding, but attenuated the response produced by a previously rewarding dose of heroin (0.1 mg/kg). Essentially, the alprazolam challenge appeared to shift the heroin-induced dose–response curve for conditioned place preferences to the left — a result consistent with the clinical reports of BDZ potentiation of opiate actions. A subsequent study examined the ability of varying doses of alprazolam (0.125–0.5 mg/kg) to alter the response to a sub-rewarding dose of IV heroin (0.25 mg/kg). The results complimented and extended the earlier findings by showing that BDZ receptor activation similarly potentiated the effects of a low dose of IV heroin, but attenuated the CPP response to heroin as the BDZ dose increased (Walker and Ettenberg, 2003). Hence it would appear that the neural substrates underlying the place preferences produced by alprazolam+heroin are equally susceptible to either opiate or BDZ receptor modulation.

One explanation or hypothesis of the mechanism by which alprazolam potentiates the behavioral effects of heroin is to assume that the two drugs are working synergistically at a common site of action. While this is by no means the only way to account for the Op–BDZ interaction, the examination of brain sites within reward systems where both opiate and BDZ drugs exert pharmacological effects seems like a reasonable place to begin the investigation of the underlying brain mechanisms responsible for the Op–BDZ interaction observed in the CPP test.

BDZ receptor agonists have been shown to exert their effects through a receptor site specific to the γ-aminobutyric acid subtype "a" ($GABA_a$) receptor–chloride ionophore complex, which is distributed throughout the brain (Hunkeler et al., 1981; Richards et al., 1986; Martin, 1987). Since we are employing a test of drug "reward" (the CPP), it would be of interest to examine brain regions known to have significant concentrations of both BDZ and opiate receptors, and thought to be involved in opiate reward. For example, the ventral tegmental area of the mammalian mesencephalon (midbrain) has been proposed as a major site for opiate reward (Phillips and LePiane, 1980; Bozarth and Wise, 1981; Bozarth, 1987). μ-opioid receptors are localized on inhibitory interneurons that, when stimulated, cause a disinhibition of dopamine (DA) neurons and a consequent increase in DA release in the nucleus accumbens (Johnson and North, 1992) — a location that is believed to play an important role in drug reward (Koob et al., 1997; De Vries and Shippenberg, 2002; Everitt and Wolf, 2002; Picciotto and Corrigall, 2002). $GABA_a$ receptors (and thus BDZ receptors) are thought to be co-localized with opiate receptors on inhibitory interneurons within the VTA (Xi and Stein, 1998) and $GABA_a$ receptor agonists administered into the VTA produce a disinhibitory effect on DA release in the nucleus accumbens (Xi and Stein, 1998). Thus, the VTA seems like a prime candidate for a prospective role in mediating the observed Op–BDZ interactions.

The present experiment was therefore devised to investigate the ability of peripheral alprazolam administration to alter place preferences produced by intra-VTA heroin infusions. This line of investigation was initiated not only to determine involvement of the mesolimbic dopamine system as a putative site for Op–BDZ interactions, but also to rule out a potential peripheral pharmacokinetic mechanism (e.g., changes in the activity of hepatic enzymes) as the basis for such interactions.

1. Methods

1.1. Animals

The subjects were 52 male Sprague–Dawley rats (each weighing 250–350 g) obtained from Charles River Laboratories (Wilmington, MA). Each rat was individually housed in hanging wire-mesh cages located within a temperature-controlled (23 °C) vivarium that was maintained on a 12-h light/dark cycle (lights on at 0700). Food and water were freely available. Upon their arrival in the vivarium, animals were gentled through daily handling over a one-week period (i.e., until surgery). The work described herein adheres to the guidelines stipulated in the 1996 NIH *Guide for the Care and Use of Laboratory Animals* and was reviewed and approved by UCSB's Institutional Animal Care and Use Committee.

1.2. Surgery

Bilateral stainless steel 22 gauge guide cannulae (Plastics Products Co., Roanoke, VA) were stereotaxically implanted under deep gas anesthesia produced by inhalation of 2% isofluorane gas. The guide cannulae were aimed at the VTA using the following coordinates (from bregma): AP −4.8, DV −7 and ML ±1.0 (Paxinos and Watson, 1986) and were secured to the skull via stainless steel screws and dental acrylic cement. Animals were returned to their home cages only after they had fully regained consciousness. Daily post-surgical oral administration of the antibiotic, enrofloxacin (4.5 mg), was utilized to prophylactically treat infection for seven consecutive days.

1.3. Apparatus

The place preference apparatus consisted of a wood-constructed rectangular enclosure measuring 156 cm long × 34 34 cm wide × 30 cm high that was divided into three chambers; two larger compartments (61 × 30 cm) at either end (one black and one white) and a middle gray zone (34 × 30 cm) separating the two. The black side of the apparatus had an acrylic (Plexiglas®) floor while the floor of the white compartment was covered with wood chip bedding that was changed prior to each trial. The middle zone had a wood floor painted gray. Prior to each trial, the black walls were wiped (5 cm from the

floor) with a cotton pad moistened with 0.1 ml of 2% acetic acid solution. This introduced a distinct olfactory cue to the black compartment that was not present in the neutral or white compartments. The apparatus, therefore, provided three distinct environments that differed in color, texture, and odor. Additionally, the dividing walls within the apparatus that separated the environments were removable so that an animal could be permitted to move freely between the three environments during place preference testing.

Fifteen infrared emitter/detector pairs were installed in the walls at approximately 10 cm intervals 2 cm above the floor along the entire length of the apparatus. The output of the detectors permitted automated determination of the animal's location within the apparatus in real time. An animal was operationally defined as being within a compartment when the number of infrared photocells interrupted in the compartment being entered was higher than the number of interrupted beams for the compartment being exited (i.e., typically requiring two-thirds of the rat's body to be within a compartment). The data were collected via a desktop computer fitted with an I/O board and running custom software.

1.4. General procedure

The experiments consisted of a ten-day experimental protocol that involved Baseline and Test place preference sessions separated by eight days of drug–place conditioning trials.

1.4.1. Baseline

On Day 1, an initial baseline was conducted in the place preference apparatus with the walls removed to provide each subject with complete access to the three test environments. Each animal was placed in the middle (gray) compartment of the apparatus and the time spent in each of the three environments was then recorded over a 15 min session. Upon completion of the trial, the animal was removed from the apparatus and returned to its home cage. The apparatus was then completely cleaned prior to the next animal's trial.

1.4.2. Conditioning trials

Days 2–9 were conditioning days in which the walls of the apparatus were in place and the animals were restricted to either the black or white compartments. On a given day, each animal received either vehicle or drug (see sections below) followed by placement into either the black or white side of the apparatus for 15 min. On the next day, the animal received the alternate treatment and was placed in the alternate environment. This continued for eight days after which each animal had experienced four drug pairings with one side of the apparatus and four saline pairings with the opposite side. The procedure was counterbalanced within each group for injection order (saline or drug) and the color of the compartment that was paired with the drug (i.e., half of the animals received drug/place pairings in their preferred environment while the other half received drug/place pairings in their non-preferred environment).

1.4.3. Test trial

Day 10 was an undrugged preference test conducted with the walls removed precisely as described for the initial baseline test.

1.5. Drugs

Diacetylmorphine HCl (heroin) was obtained from the National Institute on Drug Abuse (NIDA, Rockville, Maryland) and was prepared in a vehicle solution of nanopure H_2O. Heroin was administered in a volume of 0.5 μl per side over 84 s with the use of bilateral 10 μl syringes and 1 rpm Razel syringe pumps (Stanford, CT, Model A). To intracranially infuse the drug, a 28 gauge internal cannula was inserted into the implanted guide cannulae (bilaterally). The internal cannulae were cut so as to project 2 mm below the guide cannula into the underlying brain tissue. After infusions were completed, the cannulae were left in place for 60 s to permit drug diffusion away from the cannula tip. The benzodiazepine, alprazolam, was purchased from Sigma Pharmaceuticals (St. Louis, MO). Due to its insolubility in water, the vehicle solution consisted of 1% ethanol, 49% propylene glycol and 50% physiological saline. Alprazolam was administered IP in a volume of 1 ml/kg.

1.6. Intra-VTA heroin dose–response curve

Thirty-seven animals were randomly assigned to one of five groups ($n=7–8$/group), each corresponding to a different dose of bilateral intra-VTA heroin (2.5, 5, 10 or 40 ng as a total bilateral dose). On the conditioning days, these animals were administered an IP vehicle injection 15 min prior to a bilateral heroin infusion into the VTA. After the IC infusions, the subjects were disconnected from the drug delivery system and immediately placed into either the black or white sides of the apparatus. On alternate days, IP vehicle and intra-VTA vehicle infusions were paired with the alternate environment. Following the completion of a conditioning trial, animals were removed from the apparatus and individually transported to the vivarium.

1.7. Intra-VTA heroin + alprazolam challenge

Fifteen animals were randomly assigned to one of two groups. The first group represented an alprazolam control condition involving pairings of one environment with 0.125 mg/kg IP alprazolam + intra-VTA vehicle and pairings of IP vehicle + intra-VTA vehicle with the alternate environment ($n=7$). The second condition consisted of animals experiencing pairings of one environment with 0.125 mg/kg IP alprazolam followed by 5 ng intra-VTA heroin ($n=8$) and vehicle administrations paired with the alternate environment. As indicated above, in every case the IP injection (alprazolam or vehicle) preceded the intra-VTA infusion (heroin or vehicle) by 15 min. The final test trial, then, afforded the non-drugged subjects a choice between environments previously associated with alprazolam or alprazolam + heroin and an alternate

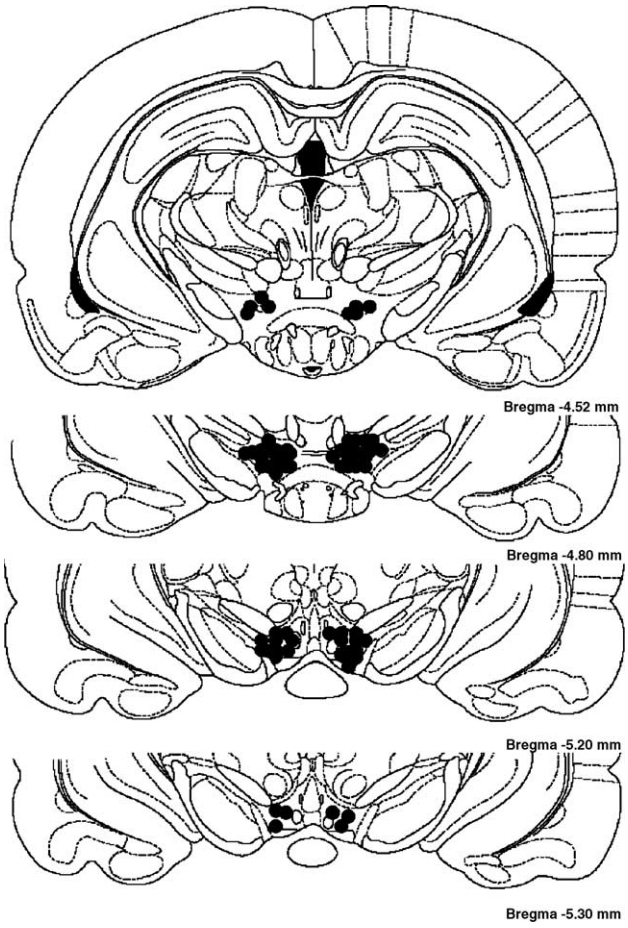

Fig. 1. Histological confirmation of infusion sites targeting the VTA. Sections adapted from Paxinos and Watson (1998).

vehicle-paired environment. The single dose of alprazolam utilized in the present experiment was previously found to potentiate the behavioral effects of IV heroin, but had no effect on place preference behavior when given alone (Walker and Ettenberg, 2001). The 5 ng dose of IC heroin was selected on the basis of results obtained in the described dose–response experiment.

1.8. Histology

Upon completion of the study, all animals were transcardially perfused with 0.9% physiological saline followed by 10% formalin solution. Brains were rapidly extracted and stored in formalin. Cannula locations were determined from 40μm frozen sections placed on slides and examined by light microscope.

1.9. Data analysis

Conditioned place preferences were operationally defined as reliable shifts from baseline to test in the time spent in a drug-paired environment following drug–place conditioning. Thus, for example, an opiate-induced place preference would be identified as an increase in the time spent on the opiate-paired side of the apparatus on test day relative to baseline. Note that in this situation, a conditioned place preference necessarily requires that an animal's test day and baseline performance be reliably different from each other. Hence, paired-sample *t*-tests (two-tailed) were conducted on each group's Baseline and Test scores. An initial ANOVA was not computed because the interest here was in determining whether or not individual conditions (group or dose) produced conditioned place preferences (shifts from baseline to test). For example, if all groups in an experiment demonstrated strong but equal-sized preferences (each group's mean difference score was reliably different from zero) then an ANOVA would still yield no statistically significant effects even though the group/dose in fact produced a CPP. Thus, the present experiment is in fact utilizing pre-planned comparisons for each group's performance on test relative to baseline (is the difference >0). Since the number of such analyses (*t*-tests) is small (ranging from two to five tests in the experiments that follow) there is little need for an adjustment to the alpha level of $p < .05$ to protect against Type I error. Indeed, such adjustments or corrections (such as the Bonferroni *t*-test) can actually be too conservative and enhance the risk of Type II errors (see Maxwell and Delaney, 1999 for a discussion of Type I and Type II errors).

2. Results

2.1. Histology

All cannulae were confirmed to be within 1 mm of the VTA (using the brain atlas of Paxinos and Watson, 1986 as a reference). The cannula tip locations are represented in Fig. 1. A representative photomicrograph of the injection sites within the VTA is presented in Fig. 2.

2.2. Baseline preferences established for CPP apparatus

The baseline preferences for all animals in the black and white compartments were 289.07 s (±20.14) and 293.69 s (±20.13) for the white and black side, respectively. This

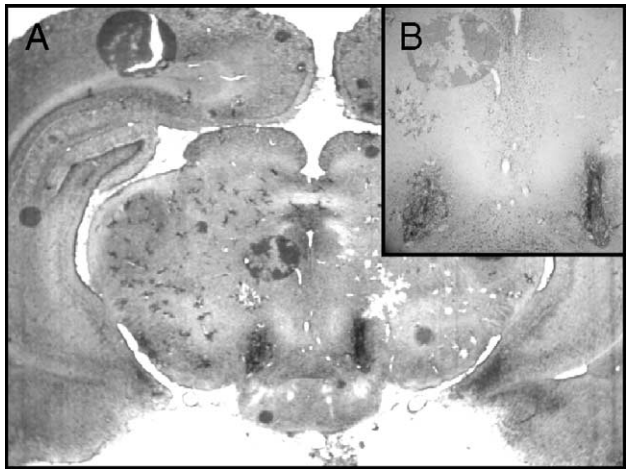

Fig. 2. Representative photomicrograph of injection sites within the VTA at 0.5× (A) and 4.0× (B) magnification. The dark vertical areas in the center of section A and magnified in section B represent the internal cannula tracks.

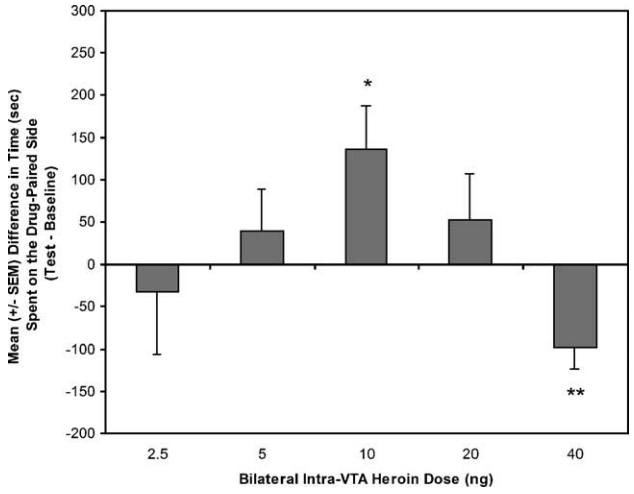

Fig. 3. Mean (±SEM) difference scores (Test–Baseline) for animals having experienced pairings of a distinctive environment with varying doses of IC heroin. Scores above or below the line, respectively, represent shifts toward or away from the drug-paired side of the apparatus on test day relative to baseline (*$p<0.05$ and **$p<0.01$).

indicates a balanced apparatus in regard to initial preference behavior.

2.3. Intra-VTA heroin dose–response curve

Fig. 3 illustrates group mean (+SEM) difference scores (Test–Baseline) for the heroin-only groups. Paired-sample t-tests confirmed that bilateral infusions of 5 ng IC heroin (10 ng total dosage) produced a highly reliable conditioned place preference ($t(7)=2.62$, $p<0.05$), while the highest dose of 40 ng resulted in learned place aversions ($t(6)=-3.85$, $p<0.01$). There was an inverted U-shaped dose–response curve across the 5 doses of intra-VTA heroin that were tested (see Fig. 3), a result that parallels our previously published data for IV heroin (Walker and Ettenberg, 2001).

2.4. Intra-VTA heroin + IP alprazolam

Fig. 4 depicts the performance of subjects receiving intra-VTA heroin (total bilateral dose equal to 5 ng) or IP alprazolam (0.125 mg/kg) by themselves or 0.125 mg/kg alprazolam + 5 ng intra-VTA heroin prior to the conditioned place preference test. Neither the heroin-only nor alprazolam-only groups showed a reliable change from zero. However, the combination of 0.125 mg/kg IP alprazolam and 5 ng intra-VTA heroin produced a reliable place preference ($t(7)=2.46$, $p<0.05$). Thus, a dose of intra-VTA heroin that itself produced no reliable conditioned place preference, when combined with a dose of IP alprazolam that similarly had no effect in the place preference test, together produced a reliable shift toward the drug-paired environment (Fig. 4).

3. Discussion

The focus of this experiment was twofold: 1) to determine whether intra-VTA heroin-induced conditioned place preferences could be established, and 2) to identify whether such preferences could be manipulated by benzodiazepine pretreatment. As illustrated in Fig. 3, an inverted U-shaped dose–response curve for intra-VTA heroin-induced place preferences was established, with the 10 ng heroin dose producing a reliable place preference ($p<0.05$). These results are in accordance with others who have demonstrated intracerebroventricular administered β-endorphin-induced place preferences (Amalric et al., 1987), intra-VTA endomorphin-1-induced (Zangen et al., 2002) and morphine-induced VTA place preferences (Phillips and LePiane, 1980; Bozarth, 1987). Additionally, the present results particularly resemble those observed with ICV β-endorphin that have been shown to produce an inverted U-shaped dose–response curve (Amalric et al., 1987). In contrast, intraperitoneal injections of heroin have been shown to produce place preferences that appear to be unrelated to the dose of heroin administered resulting in an "all or none" effect (Hand et al., 1989; Spyraki et al., 1983). Subcutaneous injections of heroin have been reported to produce an inverted U-shaped response pattern that was dose-dependent, however, the magnitude of the resulting preferences tended to be more variable within each group (Amalric et al., 1987). In a meta-analysis of opiate conditioned place preference studies, Bardo et al. (1995) reported that in the majority of studies, while opiates produce dose-dependent effects, the shape of the dose–response curve was asymptotic because place preferences were not observed to decrease from peak values with higher doses of opiates. However, this review was restricted to an examination of the effects of subcutaneous or intraperitoneal routes of opiate administration. In our previous work with intravenously administered heroin — a route of administration that removes the inherent variability in plasma absorption produced by IP or SC injections — we were able to produce a reliable dose–response curve in the CPP test. Similarly, in the present study, the direct application of heroin to the VTA also produced a robust inverted U-shaped dose–response pattern.

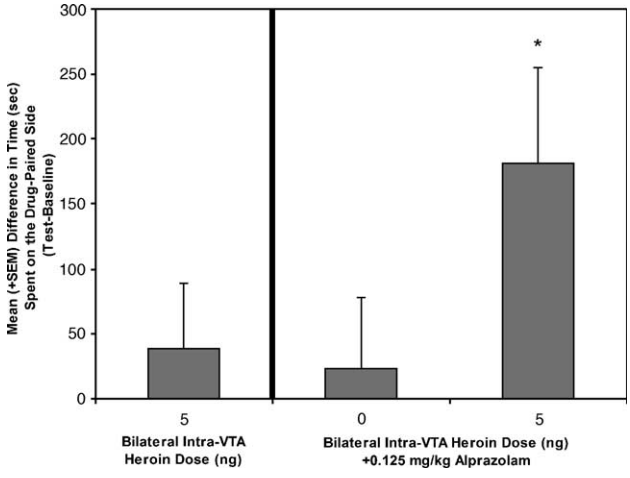

Fig. 4. Mean (±SEM) difference scores (Test–Baseline) for animals having experienced pairings of a distinctive environment with varying doses of IC heroin, IP alprazolam or IC heroin + IP alprazolam. Scores above or below the line, respectively, represent shifts toward or away from the drug-paired side of the apparatus on test day relative to baseline (*$p<0.05$).

At the highest dose tested (40 ng), a significant place aversion ($p<0.01$) was observed. Since heroin rapidly undergoes deacetylation to 6-acetylmorphine and then morphine upon passing the blood–brain barrier (Way et al., 1960; Umans et al., 1982), one possibility to account for this place aversion is the idea that although morphine binds primarily to μ-opioid receptors, at higher doses it can also bind to other types of opioid receptors, including the κ-opioid receptors (Suzuki et al., 2001). Application of κ-opioid receptor agonists has been shown to produce place aversions (Mucha and Herz, 1985) and recently has been shown to directly inhibit VTA dopaminergic neurons and produce actions that are the opposite of those produced by μ-opioid receptor activation (Margolis et al., 2003). Thus, the current aversion to the highest heroin dose might well be attributable to effects resulting from the action of morphine at κ-opioid receptors.

The fact that heroin was able to produce behavioral effects in the present study following an intracranial route of administration suggests that the enzyme responsible for the hydrolysis of heroin to 6-acetylmorphine and morphine must be present in the brain. Indeed, the particular isoform of carboxylesterase responsible for hydrolyzing heroin has been found in the vicinity of the VTA in rats (Yamada et al., 1995). Furthermore, Umans and Inturrisi (1982) have found that heroin administered via an intracerebroventricular route in rodents was a naloxone-reversible analgesic and was able to produce naloxone-sensitive respiratory depression, which supports the idea that heroin administered into the brain can affect behavior.

In our previous experiments investigating IV heroin+IP alprazolam place preference interactions (Walker and Ettenberg, 2001, 2003), it was identified that the most promising doses for reward potentiation were those that were subthreshold on the ascending CPP heroin dose–response curve. Therefore, in the present experiment, the dose of heroin (5 ng) was chosen because of its position on the intra-VTA heroin dose–response curve. The dose of alprazolam was selected to on the basis of previously thorough dose–response analyses that confirmed a strong Op–BDZ interaction without having CPP effects on its own (Walker and Ettenberg, 2001, 2003). Fig. 4 illustrates that while 5 ng of intra-VTA heroin and 0.125 mg/kg IP alprazolam were themselves non-rewarding, when combined, a significant conditioned place preference was produced ($p<0.05$). These data extend our earlier findings (Walker and Ettenberg, 2001, 2003) by demonstrating that opiate activation restricted to the VTA was sufficient to produce changes in CPPs in rats pretreated with IP alprazolam.

$GABA_a$ receptors (and thus BDZ receptors) are thought to be co-localized with opiate receptors on inhibitory GABA interneurons within the VTA (Xi and Stein, 1998). This view is supported by the reported localization of $GABA_a$ receptors in the VTA (Churchill et al., 1992) and the fact that $GABA_a$ receptor agonists administered into the VTA produce a disinhibitory effect on DA release in the nucleus accumbens (Xi and Stein, 1998). $GABA_a$ receptor activation within the VTA is therefore comparable to the effect of VTA-applied opiates (Johnson and North, 1992). It therefore seems reasonable to posit that combined Op–BDZ administration could be accounted for by additive effects of the two drugs within the VTA. Within the NAcc itself, opiate and $GABA_a$ receptor sites have also been shown to be co-localized on the dendrites of GABAergic neurons (Svingos et al., 1997). Consequently, the current CPP data suggest that the effects of alprazolam and heroin may be mediated through similar actions on a common neural system — circuitry involving the mesolimbic dopamine system.

Another possibility is that the critical site(s) where alprazolam is acting to influence heroin reward is spatially separate from opiate reward elements, but that the two regions either interact (via a common final pathway) or produce effects that sum to reach supra-threshold reward levels. For example, it is entirely possible that the benzodiazepines are operating as negative reinforcers by attenuating pre-existing levels of anxiety (and working through non-DA systems) while heroin is acting more directly to produce positive affect or euphoria through DA or endogenous opioid systems. Indeed, the amygdala has been implicated as a potential substrate for the anxiolytic actions of benzodiazepines (Menard and Treit, 1999), and particularly the basolateral amygdala, which is known to have a high density of BDZ receptors (Niehoff and Kuhar, 1983). The basolateral amygdala is of additional interest because of its anatomical connectivity with the nucleus accumbens (Setlow et al., 2002; Floresco et al., 1998; Howland et al., 2002). It could be, therefore, that alprazolam and heroin are producing their combined effects via the nucleus accumbens with one drug (alprazolam) affecting the NAcc function via the amygdala and the other (heroin) acting via the VTA. However, future studies utilizing intracranial routes of administration for both compounds must be conducted to adequately address the underlying mechanism(s) and location(s) of this interaction.

The current results add to a growing body of work on the nature of Op–BDZ interactions. For example, alprazolam has been shown to facilitate morphine-induced analgesia as measured by tail-flick latencies in laboratory rats (Bianchi et al., 1993; Pick, 1997) and potentiate the respiratory-depressant effect of dermorphin (a selective μ-opiate receptor agonist) (Paakkari et al., 1993). In this latter study, the data resembled those reported here in that alprazolam was observed to potentiate the response to a small dose of dermorphin while attenuating the response to a large dose of dermorphin. In the only other study that employed conditioned place preferences to examine BDZ+opiate interactions, Pettit et al. (1989) reported that triazolam had no effect on morphine-induced place preferences. However, in that study morphine was administered subcutaneously (not IC) and only a single morphine dose was utilized. Consistent with our results, was the fact that Pettit et al. (1989) reported that none of their five doses of triazolam produced place preferences.

The current work has considerable relevance for understanding the behavior of opiate users who self-medicate with oral benzodiazepines prior to self-administration of heroin (Stitzer et al., 1981; Preston et al., 1984; Weddington and Carney, 1987; Navaratnam and Foong, 1990a; Forsyth et al.,

1993; Iguchi et al., 1993). For example, while the data confirm that benzodiazepines can act to enhance the rewarding properties of heroin, such effects appear to be limited to the lower end of the heroin dose–response range. Thus, a relatively inexpensive treatment (e.g., alprazolam) that enhances what would otherwise be an insufficient dose of heroin could clearly represent considerable financial savings for the opiate user (Navaratnam and Foong, 1990b) which helps to explain why the concurrent use of BDZ/opiate combinations is so pervasive among opiate abusers (Navaratnam and Foong, 1990a; Iguchi et al., 1993).

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