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ScienceDirect
Pharmacology, Biochemistry and Behavior 86 (2007) 542–549
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
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Rostral–caudal differences in the effects of intra-VTA muscimol on cocaine self-administration

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Received 14 August 2006; received in revised form 11 January 2007; accepted 16 January 2007
Available online 23 January 2007

Abstract

We have found that dopamine (DA) in the ventral tegmental area (VTA) plays an important role in cocaine self-administration. DA in the VTA acts at D1-type receptors on the terminals of GABA afferents causing release of this neurotransmitter. Thus, the neurochemical pathways whereby VTA DA might be involved in cocaine self-administration may include GABA neurotransmission. In the present study, we investigated this possibility. Rats were prepared with intravenous catheters and bilateral guide cannulae positioned to allow microinjections directly into the VTA or a site 1 mm dorsal to it. The rats were then trained to self-administer cocaine (1.0 mg/kg/injection) under a fixed-ratio 1 schedule of reinforcement and tested with microinjections of muscimol (0, 0.05 and 0.1 μg/0.25 μl) or picrotoxin (0, 0.025 and 0.05 μg/0.25 μl) or trained under a progressive ratio (PR) schedule and tested with vehicle and 0.05 μg/0.25 μl muscimol. Muscimol in the VTA, but not immediately dorsal to it, significantly reduced cocaine intake under the FR1 schedule. Furthermore, when analyzed by rostral/caudal site of injection, it was found that rostral injections of muscimol significantly reduced cocaine self-administration whereas caudal injections produced non-significant decreases in self-administration. Inspection of individual records revealed no signs of non-specific behavioral effects of the muscimol treatments. Muscimol in the rostral VTA also significantly increased break points in responding under the PR schedule. Intra-VTA picrotoxin did not significantly affect cocaine self-administration. These data suggest that stimulation of GABA-A receptors in the VTA is involved in cocaine self-administration and reward and that this involvement is more pronounced in the rostral than in the caudal VTA.

Keywords: Operant responding; Reinforcement; Drug abuse; Drug addiction; Motivation; Cocaine; Self-administration; Progressive ratio; Fixed ratio

Although most research on the neural mechanisms of cocaine self-administration has focused on the role of dopamine (DA) in terminal regions of the mesocorticolimbic system, there are reports that DA neurotransmission in the cell body region of this system, as well as in the nigrostriatal system, also plays a role in cocaine self-administration. We have found that injections of a DA D1 receptor antagonist in the ventral tegmental area (VTA), the site of origin of the mesocorticolimbic DA cells, or the substantia nigra, the site of origin of the neighboring nigrostriatal DA cells, increased cocaine self-administration under fixed ratio (FR) schedules of reinforcement (Quinlan et al., 2004; Ranaldi and Wise, 2001) and decreased it under a progressive ratio schedule of reinforcement (Ranaldi and Wise, 2001). Given that D1 receptors in the VTA have been localized on non-dopaminergic afferents (Lu et al., 1997; Yung et al., 1995), the finding that stimulation of D1 receptors in this region is involved in cocaine self-administration suggests that other VTA neurotransmitters play a role in cocaine self-administration too. One possible other neurotransmitter that might be involved is gamma-aminobutyric acid (GABA).

DA D1 receptors are located on the terminals of GABA afferents to dopaminergic midbrain areas (Lu et al., 1997;Yung et al., 1995) and, when stimulated, cause GABA release (Cameron and Williams, 1993). In addition to DA cells, the VTA also contains intrinsic GABA neurons that synapse onto DA neurons (Kalivas, 1993) and GABA neurons that project to the prefrontal

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doi:10.1016/j.pbb.2007.01.017

cortex (Carr and Sesack, 2000) and nucleus accumbens (Van Bockstaele and Pickel, 1995). The VTA GABA afferents form synapses with intrinsic GABA neurons (Kalivas, 1993; Steffensen et al., 1998; Tepper et al., 1995) that themselves release GABA onto VTA DA cells, inhibiting the activity of these DA neurons, or into frontal cortex or nucleus accumbens (Carr and Sesack, 2000; Van Bockstaele and Pickel, 1995), affecting activity in those regions. Through this arrangement it is possible that VTA DA influences cocaine self-administration by activating a local pathway involving GABA release from local afferents, inhibition of intrinsic GABA neurons, disinhibition of DA cells and consequent modulation of DA release at terminals of the mesocorticolimbic pathway. Likewise, locally released GABA may influence the activity of GABA projection neurons and directly modulate activity in the terminal regions of the mesocorticolimbic system. This suggests that VTA GABA neurotransmission may be involved in cocaine self-administration.

Although there has been one investigation of the role of VTA GABA-A receptor stimulation in cocaine self-administration, this question remains unresolved. Corrigall et al. (2000) investigated the effects of muscimol, a GABA-A receptor agonist, in the VTA on cocaine self-administration in rats and found that it produced a small, but non-significant decrease in rate of cocaine intake under an FR schedule of reinforcement. In that study, muscimol was injected in what can roughly be considered the rostral half of the VTA as defined by the Paxinos and Watson (1986) rat brain atlas, and no injections were made in the caudal portion. Therefore, the role of GABA in the caudal region of the VTA remains unknown. Because of the cellular and neurochemical heterogeneity along the rostral–caudal axis of the VTA it is reasonable to hypothesize that modulation of neurotransmitter activity along this axis might affect behavior differently. Some of the heterogeneous characteristics of the VTA relevant to the role of GABA-A receptor stimulation in cocaine self-administration might include the greater number of DA neurons in the caudal than in the rostral VTA (Olson et al., 2005; Swanson, 1982) and the larger fraction of cells in the rostral VTA that are GABA-ergic than in the caudal VTA (Olson et al., 2005). Thus, it is possible that GABA in the caudal VTA plays a different—perhaps bigger or smaller—role in cocaine self-administration than does GABA in the rostral region of the VTA. In the present study we explored whether stimulation of GABA-A receptors in the rostral and caudal VTA can significantly affect cocaine self-administration. Rats were trained to self-administer cocaine under an FR schedule of reinforcement and the effects of rostral or caudal VTA injections of muscimol, the GABA-A receptor agonist, or picrotoxin, a GABA-A receptor antagonist, on rate of cocaine self-administration were investigated. Intra-VTA injections of muscimol were also investigated in rats responding for cocaine under a progressive ratio schedule of reinforcement.

1. Materials and methods

The protocols used in the present experiments are in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and have been approved by the Queens College Institutional Animal Care and Use Committee.

1.1. Subjects and surgery

Subjects were male Long Evans rats weighing between 350 and 400 g at the time of surgery. Each rat was housed individually and had free access to food (Purina rat chow) and water except during self-administration sessions when only water was available. The rats were kept on a 12/12 h light/dark cycle with the dark phase beginning at 0600 h and encompassing self-administration sessions. Each rat was anesthetized using sodium pentobarbital (65 mg/kg, i.p.) and implanted bilaterally with stainless steel guide cannulae (outer diameter = 0.635 mm; inner diameter = 0.3302 mm) so that injections could be made in the VTA or a site 1 mm dorsal to the VTA. For guide cannula implantations in the rostral VTA (ns = 19 for muscimol and 9 for picrotoxin) the flat-skull (Paxinos and Watson, 1986) coordinates were 5.6 mm caudal to bregma, ± 2.1 mm lateral to the midline (angled at 10° toward the midline in order to avoid the sagittal sinus) and 8.3 mm below the surface of the skull. For guide cannula implantations in the caudal VTA (ns = 14 for muscimol and 7 for picrotoxin) the coordinates were 6.3 mm caudal to bregma, ± 2.1 mm lateral to the midline (angled at 10° toward the midline) and 8.3 mm below the surface of the skull. For guide cannula implantations dorsal to the VTA ($n = 12$) the coordinates were 5.6 mm caudal to bregma, ± 2.1 mm lateral to the midline (angled at 10° toward the midline) and 6.3 mm below the surface of the skull. For the progressive ratio group ($n = 6$) the coordinates were those used for the rostral VTA. Obturators were placed in the guide cannulae such that they extended 1 mm beyond them and were left there at all times except during microinjections.

In the same surgical session, each rat was fitted with an indwelling jugular catheter. An incision was made in the neck and the jugular vein was isolated and opened. A silastic catheter (Dow Corning, Midland, MI) was inserted into the vein so that the tip reached a position just short of the right atrium. The catheter was tied to the vein using three sutures. The other end was fed subcutaneously to the back of the neck and exited through an opening in the back of the skull. A bent 22-guage stainless steel tube was inserted into the catheter and secured to the rat's skull with dental cement anchored by stainless steel screws. The tube served as a connector between the catheter and the drug infusion line. The catheter was flushed with heparinized saline (200 U/ml) and gentomicin (0.02 mg/ml) immediately after surgery and daily thereafter. Catheter patency was determined only when necessary—if an animal failed to learn the self-administration response or when a well-trained animal demonstrated deviations from its regular self-administration pattern—by injection of methohexital through the catheter.

1.2. Cocaine self-administration training

Five days after surgery, the animals began cocaine self-administration training. Fixed ratio 1 (FR1) schedule sessions were 2 h long and progressive ratio (PR) schedule sessions

were 5 h long. All sessions were conducted during the dark phase of the light/dark cycle. Each animal was placed in a 26×26×30 cm operant conditioning chamber equipped with a water bottle and an operant lever situated 10 cm above the floor. Each chamber had a white cue light 3 cm above the lever. Each animal was connected to a syringe in a syringe pump (Razel, 1 rpm) by polyethylene tubing through a fluid swivel. Each lever press activated the syringe pump and cue light for 14 s, causing the intravenous delivery of 1.0 mg/kg cocaine in a 0.125 ml volume of saline. This dose of cocaine was chosen because it lies well within the range of the descending limb of the cocaine dose-response curve (Gerber and Wise, 1989). On an FR1 schedule of reinforcement, decreases in responding maintained by this dose reflect leftward shifts in the cocaine dose-response function and can be interpreted as increases in the rewarding effectiveness of cocaine. During drug delivery, lever presses were counted but had no other consequence. Thus, the rats learned to lever press under an FR1 schedule of reinforcement and were tested after they achieved stable responding. Stable responding on the FR1 schedule was defined as three consecutive sessions during which the total number of infusions did not vary by greater than 10% from the mean of the three sessions. Rats met this criterion within 11 to 20 sessions. Subjects tested under the PR schedule of reinforcement were first trained under an FR1 schedule until they acquired self-administration behavior. The rats demonstrated robust self-administration by pressing the lever consistently throughout the session. Regulated responding typically resulted in approximately five self-infusions per 30-minute period, resulting in approximately 20 self-infusions over the 2-hour session. After a subject demonstrated this level of performance during a single session, it began training under a PR schedule of reinforcement in subsequent sessions. When break points (BP) in responding under the PR schedule matched stability criteria, the rats were tested with intracranial injections of muscimol. BP was operationally defined as the numerical value of the last infusion occurring within 1 h of the previous infusion. Stable BP was defined as three consecutive sessions in which the BP did not vary by more than 10% from the mean of the three sessions.

1.3. Microinjections

On test days the animals were treated with intra-VTA microinjections of muscimol (0, 0.05 and 0.1 μg/0.25 μl) or picrotoxin (0, 0.025 and 0.05 μg/0.25 μl) or muscimol (0.05 μg/0.25 μl) in a region dorsal to the rostral VTA. Immediately prior to a test session, the obturator was removed from one of the guide cannulae, and a stainless-steel injector tube (outer diameter=0.3048 mm; inner diameter=0.1524 mm) was inserted to a depth 1 mm beyond the end of the guide cannula. The injector tube was connected through polyethylene tubing to a 10-μl Hamilton micro-syringe (Reno, NV). The drug was manually injected at a rate of 0.25 μl over 30 s. The injector was kept in the guide cannula for an additional 60 s before it was removed and the obturator replaced into the guide cannula. Verification of the accuracy of microinjection volume was achieved by visual inspection of the appropriate displacement of an air bubble in the injection line. A similar microinjection was made on the contralateral side after which the rat was placed in the operant conditioning chamber and the test session was started.

Each animal was tested with either muscimol or picrotoxin and no animal was tested with more than 3 doses of either. The animals were tested with as many of the doses of muscimol or picrotoxin as possible; each test occurring on a separate day with the order of the treatment doses chosen randomly. Stable baseline responding was re-established between drug test sessions, with at least three baseline sessions between one test session and the next. If the total number of injections during the sessions following a test session did not fall within the previously established baseline and remained off baseline for more than seven sessions then the data from the recent test session were excluded from analysis. Animals were removed from the experiment if they did not meet stability criteria by the seventh session after the preceding test session or if catheters became blocked or leaky or when head assemblies became dislodged.

1.4. Histology

After the last test session, each rat was anesthetized with sodium pentobarbital, perfused with saline followed by 10% formalin, and decapitated. The brains were removed and stored in 10% formalin solution for 7 days before being cut into 40-μm serial sections and stained with cresyl violet. The sections were examined for cannula implantation and injection sites.

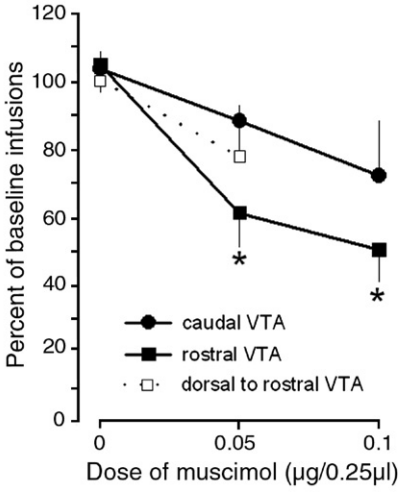

Fig. 1. Mean effects of muscimol in the rostral and caudal portions of the VTA as well as in a region dorsal to the rostral VTA (n=12) on cocaine self-administration under an FR1 schedule of reinforcement. Ns are 5, 9 and 6 for vehicle, 0.05 and 0.1 μg/0.25 μl doses in the rostral VTA, respectively and 5, 7 and 5 for vehicle, 0.5 and 0.1 μg doses in the caudal VTA, respectively. * represents significant differences from the respective vehicle condition. Vertical lines represent the standard error of the mean.

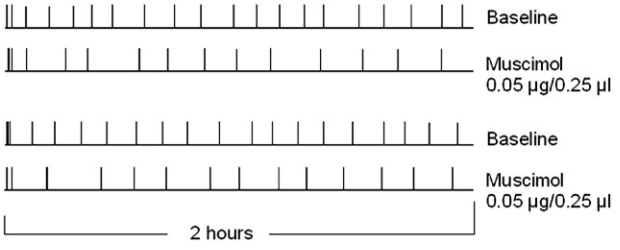

Fig. 2. Event records demonstrating self-administered injections of cocaine (1 mg/kg/injection) during 2-h sessions for 2 rats under baseline conditions and after muscimol microinjections. Each tick on the timeline represents a cocaine injection.

1.5. Data analysis

For statistical analysis animals in the FR1 experiments were assigned to rostral or caudal VTA groups. The entire VTA was defined as the VTA region that spans from the coronal planes at −4.8 to −6.8 mm posterior to bregma in the Paxinos and Watson (1986) rat brain atlas. This 2 mm distance was then divided into two equal parts to constitute the rostral and caudal VTA regions. The coronal planes extending from −4.8 to −5.8 mm posterior to bregma constituted the rostral VTA and the coronal planes extending from −6.04 to −6.8 mm posterior to bregma constituted the caudal VTA.

The total number of infusions during the test session was expressed as the percentage of the average total number of infusions for the three sessions preceding the test session. All statistical analyses were conducted on these percentages of baseline values. A separate two-way analysis of variance (ANOVA), with site of injection (rostral or caudal) and dose of test compound as factors, was conducted on the data from the muscimol and picrotoxin experiments. Because not all animals completed every treatment condition in any experiment, we used a between-subjects ANOVA model. A within-subjects model would have over-estimated statistical significance; the between-subjects model, by not factoring in the correlation between repeated test scores that existed for most of the animals tested, is a less powerful and therefore more conservative test (Keppel, 1982; Kirk, 1982). In addition to the ANOVAs, we conducted planned comparisons using *t*-tests comparing the corresponding vehicle to each dose of muscimol for each injection site. A *t*-test comparing vehicle to muscimol was conducted on the data from the dorsal control group.

BPs attained during PR test sessions were also expressed as the percentage of the average from the three sessions immediately preceding the test session. A one-way ANOVA was conducted on the data from the muscimol (0.05 µg/.25 µl) and vehicle groups.

1.6. Drugs

Cocaine hydrochloride (NIDA, Rockville, MD) was dissolved in saline. Muscimol and picrotoxin (Sigma-Aldrich, St. Louis, MO) were dissolved in artificial cerebral spinal fluid.

2. Results

The average number of cocaine infusions during the baseline sessions for all rats was 25.7 (±3.8). Microinjections of muscimol into the caudal or rostral VTA produced dose-orderly decreases in the number of cocaine infusions self-administered under an FR1 schedule of reinforcement (see Fig. 1; a 2×3 ANOVA revealed a significant dose effect $F_{(2,\ 31)}=6.688$; $p<.005$). *t*-tests showed that the rostral 0.05 [$t(12)=8.436$, $p<.01$] and rostral 0.1 [$t(9)=17.24$, $p<.005$] muscimol conditions each were significantly different from the rostral vehicle condition. When similar *t*-tests were conducted to compare the caudal 0.05 and caudal 0.1 muscimol conditions to the caudal vehicle neither comparison was significant. In the rostral group the highest dose of muscimol decreased the number of self-administered cocaine injections to less than half that seen after vehicle injections whereas in the caudal group it decreased it to less than three quarters. Event records taken from

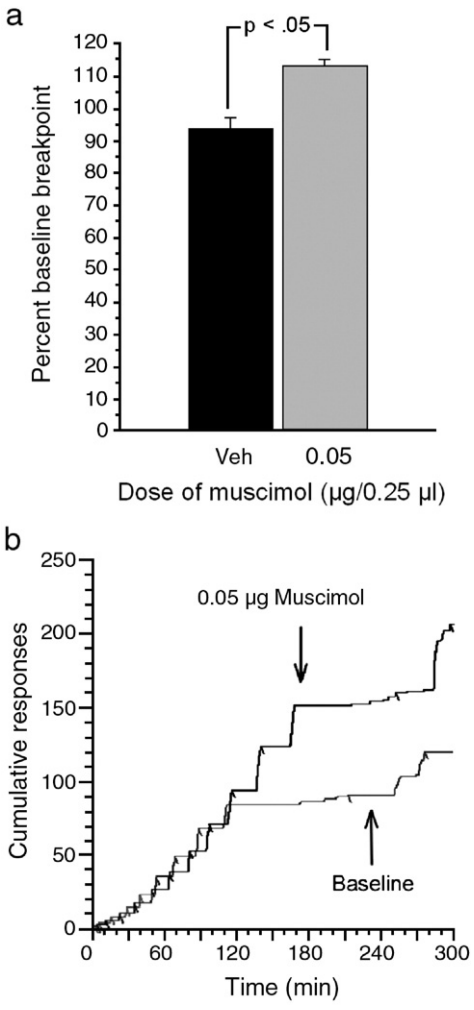

Fig. 3. (a) Mean percent of baseline break points for cocaine self-administration under a PR schedule of reinforcement after injections of vehicle or 0.05 µg/0.25 µl of muscimol ($n=6$) in the rostral VTA. Vertical lines represent the standard error of the mean. (b) Cumulative response records indicating the total number of responses and the pattern of responding for a subject in baseline (day before muscimol test) and muscimol conditions.

baseline (no injection) and muscimol treatment sessions both showed regularly-spaced injections throughout the sessions with a slower rate of intake in the muscimol condition (see Fig. 2). On average, rats self-administered a cocaine infusion approximately every 5 min under the baseline condition but only every 8 min under the highest dose muscimol condition. Microinjections of 0.05 μg/0.25 μl muscimol 1 to 2 mm dorsal to the rostral VTA did not produce changes in cocaine self-administration that were significantly different from those observed after injections of vehicle in the same region (see Fig. 1).

Microinjections of 0.05 μg/0.25 μl of muscimol in the rostral VTA significantly increased BPs under a PR schedule of reinforcement (see Fig. 3a). A one-way ANOVA comparing the BPs following vehicle injections and muscimol injections revealed a significant difference ($F_{(1,\ 8)}=5.518$; $p<.05$) between the two. Cumulative response records taken in the baseline condition showed alternations between periods of high lever pressing rates followed by post-reinforcement pauses (no lever pressing) that is typically seen during cocaine self-administration under PR schedules of reinforcement. Cumulative response records taken in the muscimol condition also showed this pattern of responding except that a greater number of injections were self-administered, ending in higher final response ratios (see Fig. 3b).

Microinjections of the 0.025 μg/0.25 μl dose of picrotoxin in the rostral VTA produced a small increase in cocaine self-administration and injections of the 0.05 μg/0.25 μl dose of picrotoxin in the caudal VTA produce a small decrease in cocaine self-administration (see Fig. 4). However, neither of these effects was significant.

Anatomical verification showed that the injection sites were reliably localized to the VTA (see Fig. 5). Microinjections aimed at the anatomical control sites were approximately 1 to 2 mm dorsal to the VTA (see Fig. 5). Tissue damage around cannula tracks and injection sites appeared similar among all animals.

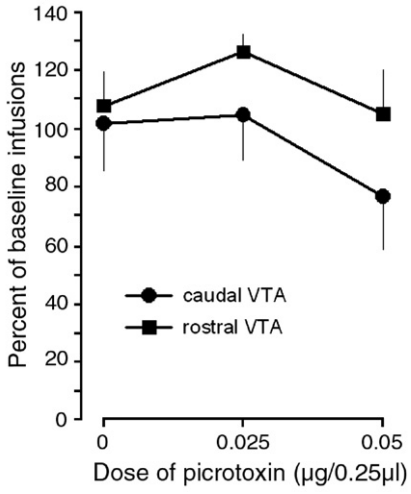

Fig. 4. Mean effects of picrotoxin in the rostral ($n=9$) and caudal ($n=7$) portions of the VTA on cocaine self-administration under an FR1 schedule of reinforcement. Ns are 4, 2 and 3 for vehicle, 0.05 and 0.1 μg/0.25 μl doses in the rostral VTA, respectively and 2, 3 and 2 for vehicle, 0.5 and 0.1 μg doses in the caudal VTA, respectively. Vertical lines represent the standard error of the mean.

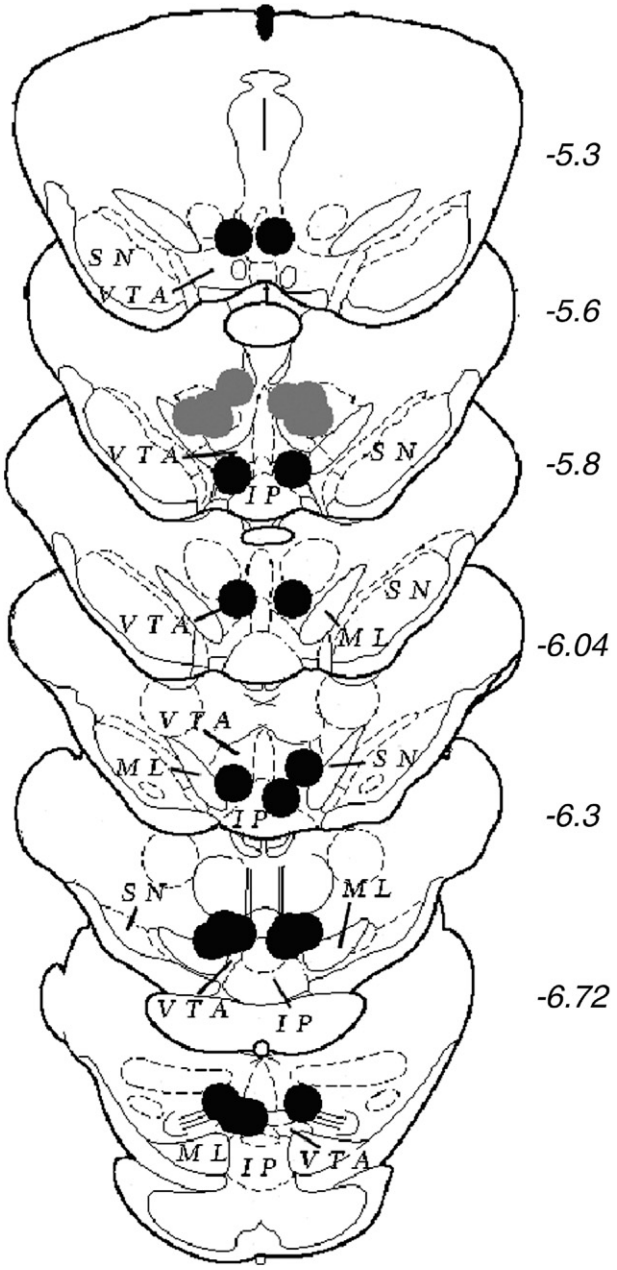

Fig. 5. Histological reconstruction of injection sites adapted from Paxinos and Watson (21). Black circles for VTA groups; grey circles for dorsal controls. Subjects were assigned to rostral or caudal groups based on whether microinjection sites were located on a plane rostral or caudal, respectively, to the −5.8 plane in the Paxinos and Watson (1986) rat brain atlas.

3. Discussion

Microinjections of muscimol into the VTA decreased rates of cocaine self-administration under an FR1 schedule of reinforcement. The pattern of self-administration seen under the muscimol conditions—regular, well-spaced infusions—was similar to that seen under baseline or vehicle conditions. The

fact that stable responding was maintained under the muscimol conditions suggests that the cocaine infusions continued to be reinforcing. However, under the muscimol conditions, the rate of self-administration was slowed. Thus, not only was cocaine still reinforcing but it appeared to satiate the animals for longer periods of time. The decreased rate of cocaine self-administration under muscimol conditions is similar to decreased rates of self-administration observed after increasing the unit dose of cocaine (Gerber and Wise, 1989). This suggests that intra-VTA muscimol changed the rewarding effects of the current dose of cocaine such that they were equivalent to those of a higher dose; in effect, intra-VTA muscimol increased the reward value of cocaine. This interpretation is supported by the increased BPs observed with the same muscimol treatment in the rostral VTA.

Microinjections of muscimol into the rostral VTA increased BPs under a PR schedule of reinforcement. Increases in BP are traditionally interpreted as increases in the rewarding effectiveness of the reinforcing stimulus (Arnold and Roberts, 1997; Hodos, 1961; Ranaldi and Wise, 2001). Thus, the increase in BPs observed here suggests that the rewarding effectiveness of the cocaine was greater under VTA muscimol treatment than not. This interpretation is consistent with the view that the decrease in FR1 responding under VTA muscimol treatment is a compensatory downward shift in drug intake caused by the greater rewarding effects of each dose (Ranaldi and Wise, 2001). Furthermore, the BP increases seen under the PR schedule argue against an interpretation that decreased rate of drug intake under the FR1 schedule are due to non-specific motoric effects. Instead, when the effects observed under both schedules are considered together, they argue for a motivational effect of VTA muscimol.

Microinjections of muscimol in a site just dorsal to the VTA did not produce significant effects on cocaine self-administration. In central injection studies it is important to ascertain that the drug effects can be accounted for by local actions of the drug and not by actions elsewhere to which the drug might diffuse. The hydraulic pressure of intracranial injections can force the injected drug into the cannula tracks (the path of least resistance) and or into pressure sinks where, in both cases, the drug can diffuse to distal sites and cause effects there. In the present study, the dorsal injections failed to affect cocaine self-administration, arguing against the possibility that the VTA injections caused their effects by diffusion of the drug in the dorsal direction.

Picrotoxin failed to produce significant effects on cocaine self-administration, whether injected into the rostral or caudal VTA. The absence of a significant picrotoxin effect should be considered cautiously. Although a significant increase in cocaine intake by picrotoxin would support the interpretation that muscimol produces its effects through GABA-A receptor actions the absence of a significant picrotoxin effect does not argue against the proposed muscimol mechanism of action nor is it contradictory to the muscimol effect. There are instances where agonists and antagonists do not produce predictably opposite effects. For instance, the role of DA D1 receptors in reward is well established (Beninger, 1993) and treatment with D1 receptor antagonists significantly reduces responding maintained by conditioned reward (Ranaldi and Beninger, 1993; Wolterink et al., 1993). This would suggest that treatment with DA D1 agonists should significantly increase this behavior. However, D1 agonists produce similar effects to antagonists; they significantly *reduce* responding for conditioned reward (Beninger and Ranaldi, 1992; Beninger and Rolfe, 1995; Ranaldi et al., 1995). These types of findings make clear that, at the systems and behavioral levels, not all physiological mechanisms in brain function in ways that we would predict from simple pharmacological suppositions.

The present results suggest that stimulation of GABA-A receptors in the VTA plays a role in cocaine self-administration and may contribute to cocaine reward. Cocaine increases DA in the VTA (Bradberry and Roth, 1989; Chen and Reith, 1994; Kalivas and Duffy, 1993; Pan et al., 1996). DA released in the VTA stimulates GABA release through actions at DA D1 receptors on the terminals of GABA afferents (Cameron and Williams, 1993). Thus, during cocaine self-administration there could occur D1 receptor-stimulated GABA release. If this D1 receptor-stimulated GABA release contributes to cocaine self-administration then one mechanism through which it does so may be stimulation of GABA-A receptors. VTA intrinsic GABA neurons have GABA-A receptors on them and stimulation of these receptors would inhibit these neurons and disinhibit DA cell activity. This would lead to modulation of terminal release of mesolimbic DA and consequently modulation of cocaine self-administration. DA cells themselves also have GABA-A receptors on them and stimulation of these would directly inhibit DA cell activity. Thus, the directional change in terminal DA concentrations would depend on the balance of GABA-A receptor-stimulated direct inhibition and indirect disinhibition of DA cell activity (Adell and Artigas, 2004). If in the present study muscimol enhanced cocaine reward by increasing DA concentrations in terminal regions, such as the nucleus accumbens, then it might be expected that intra-VTA injections of muscimol should increase DA in terminal regions. Indeed, Xi and Stein (1998) demonstrated that intra-VTA injections of muscimol can increase DA concentrations in the nucleus accumbens in rats. However, other studies have shown that intra-VTA muscimol decreases nucleus accumbens DA concentrations (Guan and McBride, 1989; Westerink et al., 1996). Thus, at present the literature is equivocal on this topic. It is important to keep in mind that in these studies the effects of intra-VTA muscimol infusions on nucleus accumbens DA were evaluated in animals that were free of the VTA neurochemical changes produced by systemic cocaine (Chen and Reith, 1994), making it even more difficult to directly relate those findings to the present study. On the other hand, intra-VTA muscimol may produce its effects on cocaine self-administration and reward not by modulating DA activity at all but instead by modulating the output of GABA projection cells. This possibility is discussed further below.

When we investigated the effects of muscimol according to site of injection—rostral or caudal VTA—we found differences. Muscimol injections in the rostral VTA produced larger decreases in cocaine self-administration than injections in the caudal VTA. Furthermore, the decreases in cocaine self-

administration after muscimol injections in the rostral VTA were significant whereas the decreases in self-administration after injections in the caudal VTA were not. This suggests that GABA actions at GABA-A receptors in the rostral VTA play a bigger role in cocaine self-administration than in the caudal VTA. Corrigall et al. (2000) tested the effects of similar doses of muscimol in a region of the VTA that overlaps with the region defined in the present study as the rostral VTA and found small, non-significant reductions in cocaine self-administration. In the present study muscimol produced larger, significant reductions in cocaine self-administration under an FR1 schedule. Among the factors that may contribute to the size difference in the muscimol effects between this and the Corrigall et al. studies may be sample size, 6 in the Corrigall et al. study and 19 in the present study, and cocaine dose, 0.3 mg/kg/inj in the Corrigall et al. study and 1.0 mg/kg/inj in the present study.

The present finding that muscimol produced differential effects based on the rostral or caudal VTA site of injection is consistent with studies demonstrating rostral–caudal heterogeneity in this region. The rostral VTA has fewer DA neurons and a greater proportion of GABA neurons than the caudal VTA (Olson et al., 2005; Swanson, 1982). How these regional differences might contribute differentially to cocaine self-administration and reward is not clear. One possibility is that the seemingly greater role of GABA-A receptor stimulation in the rostral VTA is related to the greater proportion of this region containing GABA-ergic projection neurons. These GABA cells project to the nucleus accumbens and prefrontal cortex (Carr and Sesack, 2000; Van Bockstaele and Pickel, 1995), regions highly implicated in cocaine self-administration and reward. Perhaps, muscimol produces its greater effects here because of greater modulation of GABA release in cocaine reward-related regions.

In summary, microinjections of a GABA-A agonist into the VTA reduced rates of cocaine self-administration under an FR1 schedule with larger reductions effected when injections were made in rostral versus caudal VTA. They also increased BPs for cocaine under a PR schedule. Anatomical control injections suggest that the effects of VTA injections of muscimol were local and close inspection of the data did not reveal any non-specific motoric effects. Injections of a GABA-A receptor antagonist did not significantly affect rate of cocaine self-administration regardless of its site of injection. Thus, our results suggest that stimulation of GABA-A receptors in the VTA, and more so in the rostral than in the caudal portion, plays a role in cocaine self-administration and contributes to cocaine reward.

Acknowledgment

This work was supported by a NIDA contract awarded to RR.

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