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Regional Differences Within the Rat Ventral Tegmental Area for Muscimol Self-Infusions

SATOSHI IKEMOTO,*[1] JAMES M. MURPHY† AND WILLIAM J. MCBRIDE*

Institute of Psychiatric Research, Department of Psychiatry, Indiana University School of Medicine, Indianapolis, IN, †Department of Psychology, Purdue School of Science, IUPUI, Indianapolis, IN 46202

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IKEMOTO, S., J. M. MURPHY AND W. J. MCBRIDE. *Regional differences within the rat ventral tegmental area for muscimol self-infusions.* PHARMACOL BIOCHEM BEHAV **61**(1) 87–92, 1997.—The present study examined the effects of activating $GABA_A$ receptors in the anterior and posterior regions of the ventral tegmental area (VTA) on operant reinforcement behavior, using the technique of intracranial self-administration. Rats were given the opportunity to self-administer vehicle alone (artificial CSF) and vehicle containing 25, 50, and 100 μM muscimol, a $GABA_A$ agonist, into the anterior or posterior VTA during four sessions (3 h/session) in standard two-lever operant chambers. Rats received five times greater infusions of 50 and 100 μM muscimol than vehicle into the posterior VTA; both doses significantly increased responding above vehicle levels on the active and inactive (control) levers equally. When the response requirement for muscimol infusions was increased from a fixed-ratio 1 (FR1) to FR3 in a single-lever chamber, the total session responses increased approximately twofold. Muscimol was not self-infused when cannula placements were in the anterior VTA. The self-infusion of muscimol into the posterior VTA was attenuated by coadministration of picrotoxin. Overall, the results suggest that the activation of $GABA_A$ receptors in the posterior VTA produces goal-directed behavior. © 1998 Elsevier Science Inc.

Intracranial self-administration Reinforcement Muscimol $GABA_A$ receptors Caudal linear nucleus Ventral tegmental area

THERE have been some apparent disagreements in the literature with respect to effects of GABAergic manipulations within the ventral tegmental area (VTA). One study (11) found that microinjection of $GABA_A$ agonists into the VTA increased the extracellular concentrations of dopamine (DA) and its metabolites in the nucleus accumbens. Similarly, a second study (12) reported that perfusion of muscimol into the VTA increased DA extracellular levels in the VTA. On the other hand, other investigators (7,20) found that local administration of $GABA_A$ antagonists into the anterior VTA increased DA release in the nucleus accumbens. In addition, microinjection of muscimol alone into the anterior VTA had no apparent effect on DA release in the nucleus accumbens (7).

Behavioral studies examining the effects of $GABA_A$ agonists and antagonists also reported apparent paradoxical findings. Some studies reported that microapplication of GABA or muscimol into the VTA produced heightened locomotor activity (11,12,19). On the other hand, other studies reported that microinjection of $GABA_A$ antagonists into the VTA increased locomotor activity (14,15,18), and microinjection of GABA decreased locomotor activity (9). One possibility that might explain these apparent disagreements is that there are different $GABA_A$ mediated systems between the anterior and posterior VTA. Indeed, one study (1) reported that microinjection of $GABA_A$ agonists into the posterior but not anterior VTA produced heightened locomotor activity, whereas microinjection of $GABA_A$ antagonists into the anterior but not posterior VTA produced heightened locomotor activity. Similarly, another laboratory (21) observed heightened locomotor activity after microinjection of muscimol into the posterior VTA but not into the anterior VTA. Thus, these studies suggest that different $GABA_A$ mediated neuronal mechanisms may be operating in the anterior and posterior VTA.

Recently, it was found that rats self-administered $GABA_A$ antagonists into the anterior but not into the posterior VTA (8). It is possible that rats may self-administer $GABA_A$ ago-

Requests for reprints should be addressed to Dr. W. J. McBride, Institute of Psychiatric Research, Indiana University School of Medicine, 791 Union Drive, Indianapolis, IN 46202-4887.

[1]Present address: Department of Pharmacology and Therapeutics, Louisiana State University Medical Center, 1501 Kings Highway, Shreveport, LA 71130–3932.

nists into the posterior VTA but not into the anterior VTA, because blocking $GABA_A$ receptors in the anterior VTA and activating $GABA_A$ receptors in the posterior VTA appear to produce similar effects on VTA DA neuronal activity and behavioral arousal. Therefore, the present study was designed to examine the self-administration of the $GABA_A$ agonist muscimol in the anterior and posterior regions of the VTA.

METHOD

Subjects

Subjects were adult female Wistar rats (Harlan, Indianapolis, IN) weighing 250–320 g at the time of surgery. Subjects were singly housed and maintained on a 12 L:12 D cycle (lights on during 0900–2100 h) in a temperature- and humidity-controlled environment. Food and water were freely available except during test sessions. Although not systematically studied, the estrous cycle did not appear to have a significant effect on intracranial self-administration (ICSA) behavior in the present experiments, or in previous studies, as indicated by no obvious fluctuations in ICSA behavior in rats given similar doses of the same agent for two or more sessions conducted every other day (6,8). The experimental protocols were approved by the institutional review committee and were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Chemicals

Muscimol hydrobromide (Research Biochemicals International, Natick, MA) and picrotoxin (Sigma Chemical Co., St. Louis, MO) were dissolved in artificial cerebrospinal fluid (aCSF), consisting of (in mM) 120.0 NaCl, 4.8 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 25.0 $NaHCO_3$, 2.5 $CaCl_2$, and 10.0 *d*-glucose. When necessary, pH levels were adjusted to 7.3 ± 0.2 with 0.1 N HCl.

Apparatus

An essentially identical apparatus was described previously (6,8). Briefly, the test chamber, which is situated in a sound-attenuating cubicle and illuminated by a dim house light, was equipped with either one (for Experiment 3) or two identical levers (3.5 × 1.8 cm). To minimize accidental depression of the lever by the rat brushing against them, the levers were located on a wall, 15 cm above a grid floor. In Experiments 1 and 2, when two levers were used, they were separated by 12 cm. The height of the levers required rats to rear to press the lever. The delivery of infusate was contingent on lever responses, and was controlled by a desktop computer equipped with an operant control system (L2T2 system, Coulbourn Instruments).

Microinfusions of $GABA_A$ agents were delivered by an electrolytic microinfusion transducer (EMIT) system (2,3,5). Briefly, two platinum electrodes were placed in an infusate-filled cylinder (28 mm in length × 6 mm in diameter) equipped with a 28-gauge injection cannula (Plastic One, Roanoke, VA). The electrodes were connected via spring-protected cable (Plastic One) and a swivel (Model 205, Mercotac, Inc., Carlsbad, CA) to a constant-current generator (MNC, Inc., Shreveport, LA), which delivered 4–6 μA of quiescent current and 200 μA of infusion current between the electrodes. Electrodes were acclimated to the infusate for several hours with the quiescent current before being used for testing. Fresh solutions were placed in the cylinders just prior to testing. Depression of the infusion lever delivered the infusion current for 5 s, which led to the rapid generation of H_2 gas (raising the pressure inside the gas tight cylinder) and, in turn, forced 100 nl of the infusate through the injection cannula.

Animal Preparation

Under Halothane anesthesia, a unilateral 22-gauge guide cannula (Plastic One) was stereotaxically implanted in each subject, directed to the anterior or posterior VTA. Coordinates are described below. When not in use, a 28-gauge stylet maintained the patency of the guide cannula. The stylet extended 0.5 mm beyond the tip of the guide.

At least seven days were allowed for recovery from the surgery, during which animals were brought daily to the testing room and handled for at least 5 min. Prior to beginning experimental sessions, subjects were placed in the test chamber for 30 min to acclimate them to the novel environment.

General Test Conditions

Subjects were placed individually in a test chamber. To avoid trapping air at the tip of the injection cannula, the infusion current was delivered for 5 s as the injection cannula was inserted into the guide cannula. The injection cannula extended 1.0 mm beyond the tip of the guide. For Experiments 1 and 2, a single depression of the infusion lever resulted in the delivery of 100-nl infusate over a 5-s period followed by a time-out period, during which depression of the infusion lever produced no infusion. The time-out period was given to avoid continuous delivery of infusions. For Experiments 1 and 2, the time-out period was 55 s. For experiment 3, the time-out period was reduced to 30 s. Depression of the control lever had no programmed consequence. The assignment of infusion and control levers with respect to the left and right levers was counterbalanced among subjects. For each subject, however, the assignment of infusion and control levers remained the same throughout the experiment. No cue was provided to signal the delivery or availability of infusions. No shaping technique was used to facilitate the acquisition of lever responses. The number of infusions and responses on the infusion and control levers were recorded, including during the time-out period. Experiment 3 employed a single lever and had increased response requirements.

Experiment 1: Site- and Dose-Dependent Effects of Muscimol Self-Infusions

The effect of different concentrations of muscimol to produce self-infusion behavior was evaluated in the anterior and posterior VTA, using a within-subject design. As described above, animals underwent stereotaxic surgery for implanting unilateral guide cannula. The following coordinates (16) were used (in millimeters, posterior from the bregma, lateral from the midline, ventral from the skull surface with lateral angle to the vertical): anterior VTA, 4.8, 1.5, 8.0 with a 6° angle; and posterior VTA, 6.5–7.0, 1.5, 8.0 with a 10° angle. Over four sessions, each animal was given the opportunity to respond for four different concentrations of muscimol: 0, 25, 50, or 100 μM, which produced 0, 2.5 pmol (0.5 ng), 5 pmol (1 ng), or 10 pmol (2 ng) per 100 nl infused, respectively. Testing order of these concentrations was counterbalanced among subjects.

To determine effective sites of muscimol self-infusion, the numbers of self-infusion obtained by each rat at the concentrations of 25, 50, 100 μM were added and the sum was classified into three levels: low (less than 40 infusions), medium (40 to 69 infusions), and high (70 or more infusions). Infusion sites with the infusion level for each animal are shown on

coronal drawings of the rat brain adapted from Paxinos and Watson (16). Within-subject design ANOVAs were conducted on infusion levels with the four different concentrations of muscimol for the two VTA regions separately. Within-subject design ANOVAs were also conducted on lever-response levels (infusion lever vs. control lever) with the four different concentrations of muscimol for the posterior VTA, where rats obtained significant levels of infusions.

Experiment 2: Effects of Picrotoxin on Muscimol Self-Infusions

The effects of coadministration of picrotoxin, an antagonist at the chloride channel of the $GABA_A$–benzodiazepine–chloride receptor complex, on muscimol self-infusions were examined to provide additional evidence that the self-injection of muscimol was being mediated by $GABA_A$ receptors. Six animals, which exhibited the highest self- infusion levels of muscimol into the posterior VTA in Experiment 1, were employed. The rats were given the opportunity to self-administer 50 μM muscimol alone in one session and a mixture containing 50 μM picrotoxin and 50 μM muscimol in another session; the order of testing the two solutions was counterbalanced among subjects. Picrotoxin was chosen as the antagonist because previous studies indicated that coadministration of equimolar concentrations or less of muscimol could attenuate the self-infusion of picrotoxin into the anterior VTA (8) and reduce the activation of anterior VTA DA neurons by picrotoxin (7), and that picrotoxin by itself was not self-infused into the posterior VTA at concentrations as high as 160 μM (8). A paired two-tail *t*-test was conducted on infusions with the two solutions.

Experiment 3: Effects of Increased Response Requirements on Muscimol Self-Infusions

To provide evidence for a goal-directed action for muscimol self-infusions, the effects of increasing the response requirements from fixed-ratio 1 (FR1) to FR3 were examined. The test chamber was equipped with a single lever (the right lever was removed from the chamber described above). A single lever was used in this experiment to facilitate the acquisition of response-reinforcement contingency. For the FR1 schedule, a single depression of the lever delivered an infusion of 100 nl of 100 μM muscimol over 5 s, followed by a 30-s time-out period, during which depression of the lever had no consequence. For the FR3 schedule, three depressions of the lever produced an infusion of the muscimol solution over 5 s followed by a 30-s time-out period, with the exception of the first four and second four infusions, which were respectively delivered after one and two lever presses. No cue was provided to indicate the delivery or availability of infusions. Infusions and responses during the initial FR1 and FR2 phases of the FR3 schedule were included to simplify the overall analysis. The criteria for ending the sessions with the FR1 and FR3 schedules were kept constant (sessions were terminated after 120 min, or after rats delivered 30 infusions) to more readily compare results between the two experimental paradigms.

A single guide cannula was implanted and aimed at the posterior VTA. The coordinates were −7.0 mm from bregma, lateral 1.5 mm from the midline, and ventral −8.3 mm from the skull surface with a 10° lateral angle to the vertical. The first session was an acclimation session in which animals were given the opportunity to self-administer 100 μM muscimol with the FR1 schedule. Over sessions 2 and 3, animals were tested with the FR1 and FR3 schedules; the order of testing the two schedules was counterbalanced among subjects. Paired two-tail *t*-tests were conducted between the FR1 and FR3 schedules on the number of infusions, lever responses, and time to complete the sessions.

Histology

At the conclusion of the final test session, animals were killed by CO_2 inhalation. Black India ink (0.5 μl) was injected into the site; the brain was then removed and stored at −70°C. The frozen brain was sliced into 40 μm sections, using a cryostat microtome. Sections were stained with cresyl violet. Infusion sites were examined under a light microscope.

RESULTS

Experiment 1: Site- and Dose-Dependent Effects of Muscimol Self-Infusions

Cannula placements in and around the VTA, as well as individual muscimol self-infusion levels, for 18 rats are shown in Fig. 1. Vigorous self-infusion behavior was supported when cannula placements were located in the posterior region of the VTA, particularly when near the caudal linear nucleus (CLi) of raphe. Self-infusion of muscimol was not supported in regions of the VTA anterior to −5.3 mm bregma.

For statistical analysis, animals were assigned to anterior or posterior VTA; four animals with cannula placements outside of these two VTA regions were excluded from the statistical analysis. The anterior VTA is defined as the VTA region at the level of the mammilary nuclei, coronal sections at −4.8 to −5.3 mm, as shown in Fig. 1. The posterior VTA is defined as the VTA region at the level of the CLi, and includes the CLi and the posterior portion of the parabrachial pigmented nucleus, coronal sections at −6.3 to −6.8 mm in Fig. 1.

Figure 2A shows the dose–response effects for the number of muscimol self-infusions in the anterior and posterior portions of the VTA. The posterior VTA supported significant muscimol self-infusion, $F(3, 24) = 10.14$, $p = 0.0002$. The number of infusions in the posterior VTA at the 50 and 100 μM concentrations of muscimol were fivefold higher ($p < 0.05$) than the levels attained with vehicle alone. None of the muscimol concentrations effectively maintained self-administration behavior in the anterior VTA, $F(3, 12) = 1.12$, $p = 0.4$.

Lever responses of the rats with placements in the posterior VTA were analyzed with a 2 × 4 ANOVA (Fig. 2B). Rats receiving muscimol into the posterior VTA exhibited dose-related increases in responses, $F(3, 24) = 7.02$, $p = 0.002$. However, subjects did not exhibit any preference for the infusion lever over the control lever, $F(1,8) = 2.04$, $p = 0.2$ (Fig. 2B). In addition, no lever × concentration interaction was found. In the anterior VTA, responses on the active and inactive levers were not different from each other, and were similar for vehicle and the three doses of muscimol (data not shown).

Experiment 2: Effects of Picrotoxin on Muscimol Self-Infusions

Figure 2C illustrates the effect of coadministration of 50 μM picrotoxin on the self-infusion of 50 μM muscimol into the posterior VTA. Subjects self-administered 40% fewer infusions when receiving the equimolar mixture of picrotoxin and muscimol than when receiving muscimol alone, $t(5) = 3.56$, $p = 0.02$. Coadministration of picrotoxin produced corresponding decreases in responses on the drug lever as well as the inactive (control) lever (data not shown).

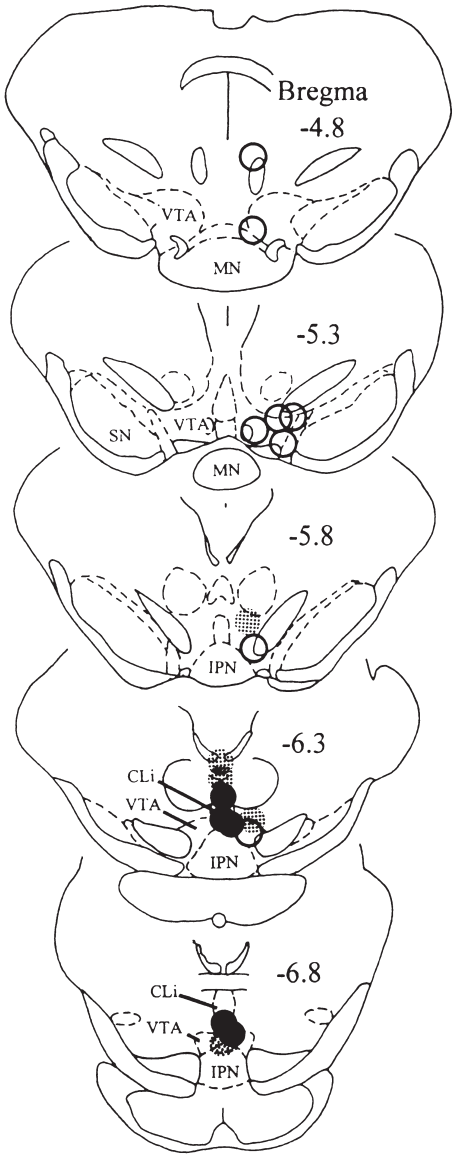

FIG. 1. Site specific effects of self-infusions of muscimol. The numbers of infusions with 25, 50, and 100 μM muscimol solutions were added for each subject (n = 18), and the sums were classified into three levels: 1–40 (low), 40–69 (medium), and 70 or more (high) infusions. Tips of injection cannulae with infusion levels of low, medium, and high are respectively indicated by empty, dotted, and black filled circles on the drawings adapted from the rat atlas of Paxinos and Watson (16). The number on the right of each coronal section indicates the distance from bregma in millimeters. CLi, caudal linear nucleus; IPN, interpeduncular nucleus; MN, mammillary nuclei; SN, substantia nigra; VTA, ventral tegmental area.

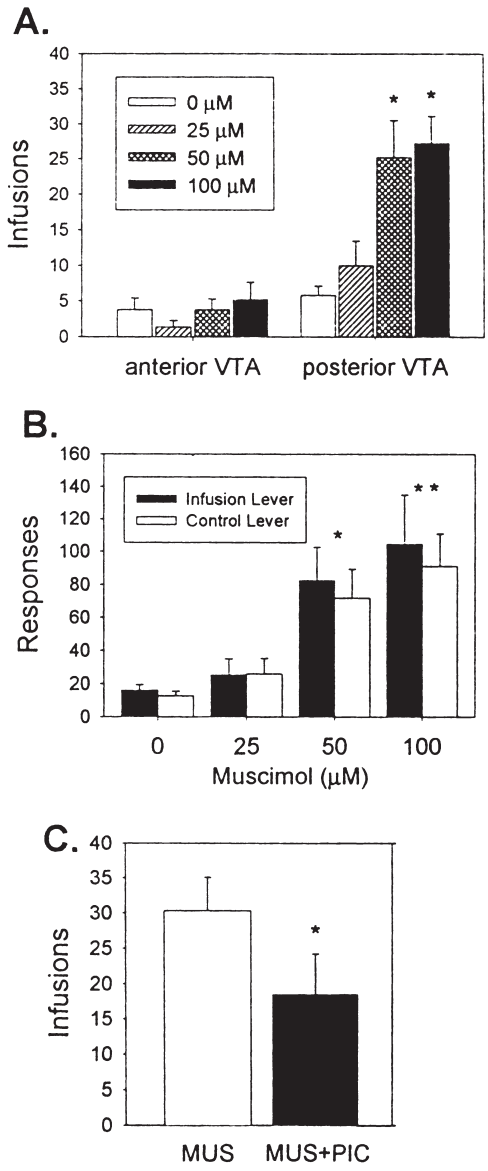

FIG. 2. (A) This shows the number of infusions of 0, 25, 50, and 100 μM muscimol into the anterior (n = 5) and posterior (n = 9) VTA. Rats were given the opportunity to respond for vehicle and the three concentrations of muscimol with a 1-min fixed-interval schedule over four sessions. Each session lasted for 3 h or was ended when rats administered 40 infusions. Data are mean ± SEM. Post hoc Scheffe F-tests revealed that in the posterior VTA, rats self-administered 50 and 100 μM muscimol more than vehicle (F = 5.53 and 6.72, respectively) or 25 μM muscimol (F = 3.39 and 4.34, respectively). *$p < 0.05$ compared to vehicle or 25 μM muscimol values. (B) Total responses on the infusion and control levers for infusions of 0, 25, 50, and 100 μM muscimol into the posterior VTA. Data are mean ± SEM. Dose-related increases in lever responses were found, *$p < 0.05$, **$p < 0.01$, compared to vehicle values. However, there were no differences in the total number of responses between the infusion and control levers at any of the muscimol concentrations. (C) Effects of coadministration of picrotoxin on the self-administration of muscimol. Rats (n = 6) were given the opportunity to self-infuse 50 μM muscimol and the solution containing 50 μM muscimol and 50 μM picrotoxin over two sessions. Rats obtained significantly lower levels of infusions when 50 μM muscimol was replaced with the mixture containing 50 μM muscimol and 50 μM picrotoxin. Data are mean ± SEM. *$p < 0.05$.

Experiment 3: Effects of Increased Response Requirements on Muscimol Self-Infusions

The effects of increasing the response requirements from FR1 to FR3 on the number of infusions, total lever responses, and time to complete the sessions are summarized in Fig. 3. Despite the increased response requirements, rats maintained nearly the same levels of infusions with the FR3 schedule (including infusions during the initial FR1 and FR2 phases) com-

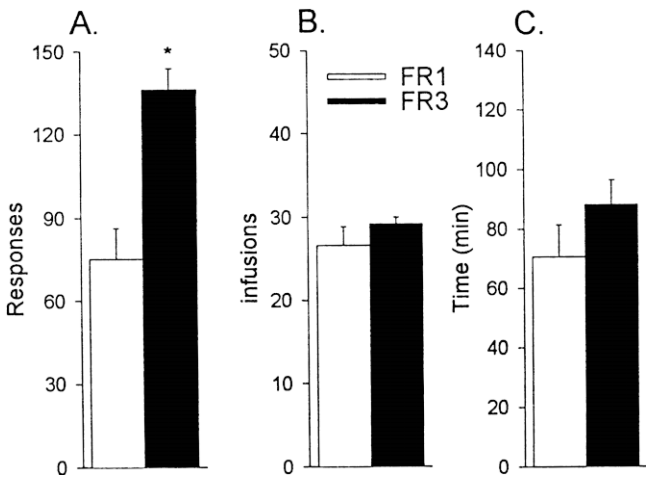

FIG. 3. Effects of increased response requirements for the self-infusion of muscimol into the posterior VTA on the total number of infusions, lever responses, and the time to complete the sessions. Rats ($n = 8$) were given the opportunity to self-administer 100 μM muscimol into the posterior VTA in a session with a FR1 schedule and another session with a FR3 schedule of reinforcement. The delivery of infusate was followed by a 30-s time-out period. Data are means ± SEM. (A) This shows the total number of responses on the single lever for muscimol during the FR1 and FR3 schedules. (B) This shows the total infusions during the FR1 and FR3 schedules. (C) This shows the time in minutes to complete the sessions for the FR1 and FR3 schedules. The two operant schedules had a differential effect on total number of lever responses (*$p = 0.001$), while the schedules did not have a reliable effect on the number of infusions ($p = 0.4$), or time to complete the sessions ($p = 0.1$).

pared to the FR1 condition, $t(7) = 0.83$, $p = 0.43$. Most of the rats, under both experimental conditions, received the maximum number of 30 infusions in less than 120 min. Some rats in both conditions received the 30 infusions in less than 60 min. Thus, the mean total number of infusions was nearly 30 for both experimental situations; therefore, the times to complete the sessions for the two schedules were considerably less than 120 min (Fig. 3C). To maintain comparable infusion under the two schedules, rats responded significantly more during the FR3 schedule than with the FR1 schedule, $t(7) = 5.06$, $p = 0.001$. In addition, the times to complete the sessions were not significantly different between the two schedules, $t(7) = 1.88$, $p = 0.10$, although there was a trend for the rats to take more time to complete the session with the FR3 schedule.

DISCUSSION

The present results suggest that activation of $GABA_A$ receptors in the posterior VTA may produce a reinforcing effect. Self-administration behavior was supported by microinfusion of muscimol into the posterior VTA (Figs. 1 and 2), whereas muscimol was not self-infused into the anterior VTA (Figs. 1 and 2A). When picrotoxin was coadministered with muscimol, self-infusion behavior was substantially attenuated (Fig. 2C), providing additional evidence that the effects of muscimol were mediated by $GABA_A$ receptors.

A major issue of the present study is whether muscimol infusions into the posterior VTA are reinforcing, or merely a result of general arousal. Although rats readily self-infused the 50 and 100 μM muscimol solutions into the posterior VTA, they did not show preference for the lever that produced infusions compared to the lever without any programmed consequence (Fig. 2B). The nearly equal amount of responding on both the control and infusion levers suggests that muscimol may be producing general arousal. Previous studies have documented that microinjection of muscimol into the VTA produces a general enhancement in motor activity (1,11,21), which was characterized as compulsive walking without exploration (1). However, the lack of lever discrimination may be due, in part, to a generalization effect (i.e., the two levers were physically indistinguishable from each other), and to rats not receiving sufficient training to discriminate the infusion from the control lever (i.e., subjects received doses that supported self-infusion behavior in only two of the four sessions). A lack of lever discrimination was also seen, using similar experimental manipulations, for agents that subsequently demonstrated reinforcing effects with other tests (6,8). Furthermore, it is unlikely that simple arousal could lead to reliable lever responding, because the levers were located at a height which minimized their accidental depression by general motor activity. Moreover, the results of Experiment 3 (Fig. 3) provide additional evidence that the increased infusions of 50 and 100 μM muscimol were not solely a result of increased general arousal. When the requirement for the delivery of infusions was increased from FR1 to FR3, rats maintained their infusion levels by satisfying the increased response demands. The increased responding to maintain infusion levels within the same time period cannot be explained by the notion that muscimol infusions simply produced a nondirected arousal, because the simple arousal hypothesis predicts decreased infusion levels and, thereby, decreased lever responding, when requirements for the delivery of infusions are raised. Therefore, the present findings are consistent with an interpretation that muscimol infusions into the posterior VTA produces goal-directed behavior.

It is not clear whether the effects of muscimol on operant responding can be attributed solely to its action in the CLi, or if other nuclei might also be involved or be mainly responsible for the actions of muscimol (Fig. 1). The present results suggest that the CLi might play a major role in supporting muscimol self-infusion behavior. However, it is possible that the posterior regions of the parabrachial pigmented nucleus (which constitutes the major part of the VTA) and the interpeduncular nucleus (immediately ventral to the VTA) could also be involved, or have a major role in supporting muscimol self-administration. Further investigations are needed to provide more definitive evidence. Thus, at the present time, the anterior and posterior distinction is useful for discussing regionally different GABA mechanisms mediating VTA functions.

The present study suggests that there are differences between the anterior and posterior VTA in neuronal mechanisms mediating operant responding behavior. The study indicates that activating $GABA_A$ receptors in the posterior VTA enhances operant responding, whereas activating $GABA_A$ receptors in the anterior VTA, by itself, does not increase operant behavior (Figs. 1 and 2). These data are compatible with a previous study (8), which demonstrated that rats would self-administer $GABA_A$ receptor antagonists into the anterior VTA, whereas self-administration behavior was not supported when the antagonist was offered in the posterior VTA. The differences observed in self-infusion of the $GABA_A$ agonist and antagonist between the anterior and posterior VTA might be due to differences in GABA neuronal mechanisms regulating the activity of DA neurons within the two subregions. Microdialysis studies suggested that blocking $GABA_A$ receptors in the anterior VTA resulted in activation of DA

neurons (7,20). One possible explanation for this activation is that the antagonists are blocking tonic $GABA_A$-receptor mediated inhibition of DA neurons. On the other hand, in the posterior VTA, activating $GABA_A$ receptors may increase the activity of DA neurons by inhibiting GABA interneurons (10–12). Therefore, there may be a relationship between the activating effects of the $GABA_A$ agents on VTA DA neuronal activity and the effects that these agents have on supporting operant reinforcement behavior. Furthermore, the overall results of the present study, and those previously published, support the hypothesis that different neuronal mechanisms, which are involved in mediating operant reinforcement, are functioning within the anterior and posterior regions of the VTA.

Under limited access paradigms, low doses of competitive antagonists increase the IV self-administration of the opiates and psychomotor stimulants, whereas higher doses, or doses of noncompetitive antagonists, tend to produce decreases in self-administration behavior [reviewed in (13)]. In the present study, coadministration of an equimolar concentration of picrotoxin with muscimol reduced the number of self-infusions approximately 40% (Fig. 2C). This result is in agreement with other intracranial self-administration (ICSA) studies (4,5). Equimolar concentrations of sulpiride reduced the ICSA of cocaine into the medial prefrontal cortex (5), and systemic administration of sulpiride reduced the self-infusion of bicuculline into the VTA of mice (4). Contrary to the above ICSA results with antagonists, an equimolar concentration of sulpiride and amphetamine produced the same responses/min as amphetamine alone, whereas coinfusion of a lower dose of sulpiride enhanced amphetamine responding approximately 20% (17). Therefore, in the present study, decreased, and not increased, responding was observed with the addition of picrotoxin to the muscimol solution because of the high antagonist concentration and/or the noncompetitive interaction of picrotoxin and muscimol at the $GABA_A$ receptor complex.

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REFERENCES

1. Arnt, J.; Scheel-Krüger, J.: GABA in the ventral tegmental area: Differential regional effects on locomotion, aggression and food intake after microinjection of GABA agonists and antagonists. Life Sci. 25:1351–1360;1979.
2. Bozarth, M. A.; Wise, R. A.: Electrolytic microinfusion transducer system: An alternative method of intracranial drug application. J. Neurosci. Methods 2:273–275; 1980.
3. Criswell, H. E.: A simple chronic microinjection system for use with chemitrodes. Pharmacol. Biochem. Behav. 6:237–238; 1977.
4. David, V.; Durkin, T. P.; Cazala, P.: Self-administration of the $GABA_A$ antagonist bicuculline into the ventral tegmental area in mice: Dependence on D_2 dopaminergic mechanisms. Psychopharmacology (Berlin) 130:85–90; 1997.
5. Goeders, N. E.; Smith, J. E.: Intracranial self-administration methodologies. Neurosci. Biobehav. Rev. 11:319–329; 1987.
6. Ikemoto, S.; Glazier, B. S.; Murphy, J. M.; McBride, W. J.: Role of D_1 and D_2 receptors in the nucleus accumbens in mediating reward. J. Neurosci. 17:8580–8587; 1997.
7. Ikemoto, S.; Kohl, R. R.; McBride, W. J.: $GABA_A$ receptor blockade in the anterior ventral tegmental area increases extracellular levels of dopamine in the nucleus accumbens of rats. J. Neurochem. 69:137–143; 1997.
8. Ikemoto, S.; Murphy, J. M.; McBride, W. J.: Self-infusion of $GABA_A$ antagonists directly into the ventral tegmental area and adjacent regions. Behav. Neurosci. 111:369–380; 1997.
9. Ikemoto, S.; Panksepp, J.: Dissociations between appetitive and consummatory responses by pharmacological manipulations of reward-relevant brain regions. Behav. Neurosci. 110:331–345; 1996.
10. Kalivas, P. W.: Neurotransmitter regulation of dopamine neurons in the ventral tegmental area. Brain Res. Rev. 18:75–113; 1993.
11. Kalivas, P. W.; Duffy, P.; Eberhardt, H.: Modulation of A10 dopamine neurons by gamma-aminobutyric acid agonists. J. Pharmacol. Exp. Ther. 253:858–866; 1990.
12. Klitenick, M. A.; DeWitte, P.; Kalivas, P. W.: Regulation of somatodendritic dopamine release in the ventral tegmental area by opioids and GABA: An in vivo microdialysis study. J. Neurosci. 12:2623–2632; 1992.
13. Koob, G. F.; Weiss, F.: Pharmacology of drug self-administration. Alcohol 7:193–197; 1990.
14. Mogenson, G. J.; Wu, M.; Jones, D. L.: Locomotor activity elicited by injections of picrotoxin into the ventral tegmental area is attenuated by injections of GABA into the global pallidus. Brain Res. 191:569–571; 1980.
15. Mogenson, G. J.; Wu, M.; Manchanda, S. K.: Locomotor activity initiated by microinjection of picrotoxin into the ventral tegmental area. Brain Res. 161:311–319; 1979.
16. Paxinos, G.; Watson, C.: The rat brain in stereotaxic coordinates, 2nd ed. San Diego; Academic Press Inc.; 1986.
17. Phillips, G. D.; Robbins, T. W.; Everitt, B. J.: Bilateral intra-accumbens self-administration of *d*-amphetamine: Antagonism with intra-accumbens SCH-23390 and sulpiride. Psychopharmacology (Berlin) 114:477–485; 1994.
18. Stinus, L.; Herman, J. P.; Le Moal, M.: GABAergic mechanisms within the ventral tegmental area: Involvement of dopaminergic (A10) and nondopaminergic neurons. Psychopharmacology (Berlin) 77:186–192; 1982.
19. Tanner, T.: GABA-induced locomotor activity in the rat, after bilateral injection into the ventral tegmental area. Neuropharmacology 18:441–446; 1979.
20. Westerink, B. H. C.; Kwint, H.-F.; deVries, J. B.: Neuropharmacology of mesolimbic dopamine neurons: Dual-probe microdialysis study in the ventral tegmental area and nucleus accumbens of the rat brain. J. Neurosci. 16:2605–2611; 1996.
21. Wirtshafter, D.; Klitenick, M. A.: Comparative studies of locomotor behavior following microinjections of muscimol into various sites in the paramedian tegmentum. Pharmacol. Biochem. Behav. 32:625–628; 1989.