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Intracranial Self-Administration of Cocaine within the Posterior Ventral Tegmental Area of Wistar Rats: Evidence for Involvement of Serotonin-3 Receptors and Dopamine Neurons

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ABSTRACT

The rewarding properties of cocaine have been postulated to be regulated, in part, by the mesolimbic dopamine (DA) system. The present study assessed whether adult female Wistar rats would self-administer cocaine directly into the VTA. Following guide cannulae surgery aimed at either the posterior or anterior VTA, subjects were placed in an operant box equipped with an 'active lever' that caused the delivery of the infusate and an 'inactive lever' that did not. Posterior and anterior VTA subjects were randomly assigned to one of six groups that self-administered either artificial CSF (aCSF) or 25 to 400 pmol cocaine/100 nl in aCSF for the first four sessions, aCSF in sessions 5 and 6, and the acquisition dose of infusate during session 7. Additionally, the effects of increasing the 'time-out' period, higher concentrations of cocaine, co-administration of a $5HT_3$ antagonist, and co-administration of a $D_{2/3}$ agonist on self-infusion of cocaine were determined. Self-infusions were maintained when the 'time-out' period was extended from 5 to 25 sec. Co-infusion of a $5HT_3$ antagonist or $D_{2/3}$ agonist blocked the self-infusion of cocaine. In contrast, rats did not self-administer 25-400 pmol/100 nl cocaine into the anterior VTA. Additionally, rats did not self-administer either 800 or 1600 pmol/100 nl cocaine into the posterior or anterior VTA. Overall, the data indicate that the VTA is functionally heterogeneous with regard to the rewarding actions of cocaine, and that the reinforcing effects of cocaine within the posterior VTA are mediated by activation 5-HT_3 receptors and DA neurons.

The intracranial self-administration (ICSA) technique has been used to reliably identify specific brain regions involved in the initiation of response-contingent behaviors for the delivery of a reinforcer (Bozarth and Wise, 1980; Goeders and Smith, 1987; McBride et al., 1999). Previous ICSA research indicated that cocaine was self-administered into the medial prefrontal cortex (mPFC; 50-90 pmol), but not in the nucleus accumbens (Acb) or the ventral tegmental area (VTA) (Goeders and Smith, 1983). Additional research reported that cocaine self-administration in the mPFC was blocked by presynaptic lesioning of dopamine (DA) neurons, or co-administration of the D_2 receptor antagonist sulpride (Goeders and Smith, 1986). Thus, cocaine self-administration in the mPFC was dependent upon an intact DA system and activation of D_2 receptors (Goeders and Smith, 1986). Recently, our laboratory reported that cocaine was self-administered into the Acb shell (AcbSh), but not the Acb core (AcbC), between concentrations of 400-1200 pmol/100 nl (Rodd-Henricks et al., 2002b). A similar regional heterogeneity within the Acb was reported for the dopamine reuptake blocker nomifensine (Carlezon et al., 1995). Additionally, we reported that co-administration of the $D_{2/3}$ receptor antagonist sulpiride inhibited cocaine self-infusion in the AcbSh (Rodd-Henricks et al., 2002b).

Although not initially found to support cocaine self-administration (Goeders and Smith, 1983), activation of the VTA has been shown to mediate some of the effects of systemically administered cocaine. Activation of the VTA has been postulated to be a critical part of cocaine reinforcement (Spanagel and Weiss, 1999). In humans, fMRI studies have linked activation of the VTA with the subjective rating of euphoria, and may be involved in the 'rush' experienced following cocaine administration (Breiter et al., 1997; Breiter and Rosen, 1999). In rats, infusion of a D1 antagonist into the VTA inhibited

intravenous cocaine self-administration (Ranaldi and Wise, 2001). Microinjection of glutamate antagonists within the VTA can block the development of cocaine-induced condition place preference (Harris and Aston-Jones, 2003). Similarly, electrophysiological studies indicate that perfusion of midbrain slices with cocaine can augment invoked firing rates of VTA DA neurons (Bunny et al., 2001). Specifically, Bunny et al. (2001) reported that cocaine, and the ethanol (EtOH)/cocaine metabolite cocaethylene, could enhance EtOH-induced VTA DA neuronal firing. A number of studies have indicated that EtOH is self-administered into the posterior VTA (Gatto et al., 1994; Rodd-Henricks et al., 2000, 2003; Rodd et al., 2004a-c) whereas the anterior VTA does not support EtOH self-administration (Rodd et al., 2004b, c; Rodd-Henricks et al., 2000). The results with EtOH suggest differences in functional activity exist between the anterior and posterior VTA with regard to reinforcement. Studies by Ikemoto et al. (1997b, 1998) also indicated differences exist between the anterior and posterior VTA with regard to $GABA_A$ receptor mechanism mediating reinforcement. Anatomically, the VTA is also a heterogeneous structure composed of five identified nuclei (for review see Oades and Halliday, 1987). Analysis of the cytoarchitecture of the VTA has revealed that the VTA contains morphologically distinct dopaminergic cells that display specialized axonal projections (Phillipson, 1979) and topographic afferent and efferent projections (Kalen et al., 1988; Tan et al., 1995; Brog et al., 1993).

In the initial cocaine VTA ISCA study (Goeders and Smith, 1983), the locations of the injection sites were not analyzed in detail, although the surgical coordinates suggests that placements were primarily in the anterior VTA. The studies cited above indicate functional

and anatomical heterogeneity with the VTA. Therefore it is possible that the reinforcing effects of cocaine may depend upon the injection sites within the VTA.

The present study was undertaken to re-examine cocaine response-contingent behaviors within the VTA to determine (a) whether the anterior or posterior VTA would support ICSA of cocaine; and (b) if cocaine is self-infused whether activation of DA neurons and local 5-HT3 receptors is involved. To test the involvement of DA neurons, the co-infusion of a D2/3 agonist, quinpirole, was used. Local administration of quinpirole into the VTA reduces DA cell firing rates (Congar et al., 2002; Jeziorski and White 1989). The actions of cocaine appear to be mediated in part through activation of 5-HT3 receptors (King et al., 1995, 2000, 2002), and the activity of VTA DA neurons appears to be regulated by 5-HT3 receptors (Campbell et al., 1996; Minabe et al., 1991; Rasmussen et al., 1991). Therefore, the involvement of 5-HT3 receptors in cocaine self-infusion into the VTA was tested by co-administration of a 5-HT3 receptor antagonist. The hypothesis to be tested is that there is regional heterogeneity within the VTA, involving activation of DA neurons, for the self-infusion of cocaine.

Methods

Animals

Naïve, female Wistar rats (Harlan, Indianapolis, IN) weighing 250-320 g at time of surgery were used. Animals were double-housed upon arrival and maintained on a 12-hr reverse light-dark cycle (lights off at 0900 hr). Although not systematically studied, the estrus cycle did not appear to have a significant effect on ICSA behavior in the present study, or in previous studies (Gatto et al., 1994; Ikemoto et al., 1997a, b, 1998; Rodd-Henricks et

al., 2000, 2002a, b, 2003; Rodd et al., 2003, 2004a-c), 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. The main benefit of using female rats is that the success rate of survival and correct injector location is higher in females (~ 95%) than males (~80-85%) rats. For EtOH self-administration into the posterior VTA, the results indicated that male and female Wistar rats were similar (Rodd et al., 2004b). Food and water were freely available except in the test chamber. All research protocols were approved by the institutional animal care and use committee and are in accordance with the guidelines of the Institutional Care and Use Committee of the National Institute on Drug Abuse, NIH, and the Guide for the Care and Use of Laboratory Animals (National Research Council 1996).

Data for rats that did not complete all experimental test sessions were eliminated from the analyses. The number of animals indicated for each experiment represents approximately 98% of the total number that underwent surgery; 2% of the animals were not included for analyses mainly due to the loss of the guide cannula before completion of all experimental sessions. The data for these animals were not used because their injection sites could not be verified.

Drug and Vehicle

The artificial cerebrospinal fluid (aCSF) vehicle consisted of 120.0 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM Mg SO_4, 25.0 mM $NaHCO_3$, 2.5 mM $CaCl_2$, and 10.0 mM d-glucose. Cocaine HCl (NIDA), quinpirole (Sigma) and ICS 205,930 (Sigma) were dissolved in the aCSF solution. When necessary, 0.1 M HCl or 0.1 M NaOH was added to the solutions to adjust pH levels to 7.4 ± 0.1.

Apparatus

The test chambers (30 x 30 x 26 cm; w x h x d) were situated in sound-attenuating cubicles (64 x 60 x 50 cm, Coulbourn Instruments, Allentown, PA) which were illuminated by a dim house-light during testing. Two identical levers (3.5 x 1.8 cm) were mounted on a single wall of the test chamber, 15 cm above a grid floor, and were separated by 12 cm. Levers were raised to this level to avoid accidental brushing against the lever and to reduce responses as a result of locomotor activation. Directly above each lever was a row of three different colored cue lights. The light (red) to the far right over the active bar was illuminated during resting conditions. A desktop computer equipped with an operant control system (L2T2 system, Coulbourn Instruments) recorded the data and controlled the delivery of infusate in relation to lever response.

An electrolytic microinfusion transducer (EMIT) system (see Bozarth and Wise, 1980) was used to control microinfusions of drug or vehicle. Briefly, two platinum electrodes were placed in an infusate-filled cylinder container (28 mm in length x 6 mm in diameter) equipped with a 28-gauge injection cannula (Plastics One, Roanoke, VA). The electrodes were connected by a spring-coated cable (Plastics One) and swivel (Model 205, Mercotac, Carlsbad, CA) to a constant current generator (MNC, Shreveport, LA) that delivered 6 uA of quiescent current and 200 uA of infusion current between the electrodes. Depression of the active lever delivered the infusion current for 5 sec, which led to the rapid generation of H_2 gas (raising the pressure inside the airtight cylinder), and, in turn, forcing 100 nl of the infusate through the injection cannula. During the 5-sec infusion and additional 5-sec timeout period, the house light and right cue light (red) were extinguished and the left cue light (green) over the active lever flashed on and off at 0.5 sec intervals.

Animal Preparation

While under isoflurane anesthesia, a unilateral 22-gauge guide cannula (Plastics One) was stereotaxically implanted in the right hemisphere of each subject, aimed 1.0 mm above the target region. Coordinates for placements into the posterior VTA were 5.4 to 6.0 mm posterior to bregma, 2.1 mm lateral to the midline, and 8.5 mm ventral from the surface of the skull at a 10° angle to the vertical. Coordinates for placements into the anterior VTA were 4.8 to 5.2 mm posterior to bregma, 2.1 mm lateral to the midline, and 8.5 mm ventral from the surface of the skull at a 10° angle to the vertical. The delineation of anterior/posterior VTA is based on neuroanatomical data that indicates that serotonin innervation is evident posterior to – 5.3 mm bregma (Herve et al., 1987), but is not observed anterior to -5.3 mm bregma. Additionally, segmenting the VTA at approximately -5.3 mm bregma is consistent with neuroanatomical data associated with differences in dopaminergic cells morphology (Phillipson, 1979) and topographic afferent and efferent projections (Kalen et al., 1988; Tan et al., 1995; Brog et al., 1993). In between experimental sessions, a 28-gauge stylet was placed into the guide cannula and extended 0.5 mm beyond the tip of the guide. Following surgery, all rats were individually housed and allowed to recover 7-10 days. Animals were handled for at least 5 min daily following the fourth recovery day. Subjects were not acclimated to the test chamber prior to the commencement of data collection, nor were they trained on any other operant paradigm.

General Test Condition

For testing, subjects were brought to the testing room, the stylet was removed, and the injection cannula screwed into place. Rats were placed individually in the test chamber. To avoid trapping air at the tip of the injection cannula, the infusion current was delivered for 5

sec during insertion of the injector that resulted in a non-contingent administration of cocaine or aCSF at the beginning of the session. Injection cannulae extended 1.0 mm beyond the tip of the guide. The test chamber was equipped with two levers. Depression of the 'active lever' (FR1 schedule of reinforcement) caused the delivery of a 100-nl bolus of infusate over a 5-sec period followed by a 5-sec time-out period. During both the 5-sec infusion period and 5 sec time-out period, responses on the active lever did not produce further infusions. The assignment of active and inactive lever with respect to the left or right position was counterbalanced among subjects. However, the active and inactive levers remained the same for each rat throughout the experiment. No shaping technique was used to facilitate the acquisition of lever responses. The number of infusions and responses on the active lever was recorded. Responses on the 'inactive lever' were recorded, but did not result in infusions. The duration of each test session was 4 hr and sessions occurred every other day.

Dose Response

For the posterior VTA, animals were randomly assigned to one of six groups (n = 6-10/group; total n = 53). A vehicle group received infusions of aCSF for all seven sessions. The other groups received infusions of 25, 50, 100, 200, or 400 pmol/100 nl (0.25-4.0 mM) cocaine for the first four sessions. During the fifth and sixth sessions, all animals received infusions of aCSF. On the seventh session, rats were allowed to respond for their originally assigned infusate. A total of 53 rats completed the training procedure. In addition, 5 rats from various cocaine groups (1 with 50 pmol/100 nl, 2 with 100 pmol/100 nl, and 1 with 200 pmol/100 nl) had placements outside the posterior VTA. These subjects were collapsed across infusate groups and served as neuroanatomical controls. For the anterior placements, animals were randomly assigned to one of six groups (n = 6-8/group; total n = 34), which

were treated the same as the posterior VTA group and received the same concentrations of cocaine. Only 2 rats had placements outside the anterior VTA (100 and 200 pmol/100 nl). Overall, the survival rate of rats in all experiments was nearly 96%.

Higher Cocaine Concentrations

Previously, we reported that Wistar rats would self-administer 400-1200 pmol/100 nl directly into the AcbSh (Rodd-Henricks et al., 2002b). The initial dose-response experiment indicated that cocaine concentrations as high as 4 mM were not self-infused into the anterior VTA. Therefore we examined the possibility that higher cocaine concentrations might be self-infused into the anterior VTA. For comparison purposes, these higher concentrations of cocaine were also tested in the posterior VTA. Rats with guide cannulae implanted in either the posterior (n = 8) or anterior (n = 8) VTA were allowed to self-infuse 800 pmol/100 nl cocaine for the initial four sessions, and were then allowed to self-infuse 1600 pmol/100 nl cocaine for the last four sessions.

Extended Time-Out

An additional control experiment was conducted to determine if extending the time-out period between infusions would alter self-administration of cocaine into the posterior VTA. A number of psychostimulants can induce stereotypy, also called reflexive responding under operant test conditions (Goeders and Smith, 1987). Therefore, a recommended control experiment is to extend the inter-infusion-interval by increasing the time-out period, which would prevent repetitive movements induced by the psychostimulant from producing operant responding (Goeders and Smith, 1987). Thus, during the initial 4 sessions, depression of the 'active lever' (FR1 schedule of reinforcement) caused the delivery of a 100-nl bolus of infusate over a 5-sec period followed by a 5-sec time-out period. During the last 4 sessions,

depression of the 'active lever' (FR1 schedule of reinforcement) caused the delivery of a 100-nl bolus of infusate over a 5-sec period followed by a 25-sec time-out period. During both infusion timeout periods, responses on the active lever were recorded but did not produce further infusions. Responses on the 'inactive lever' were recorded, but did not result in infusions. Under these conditions, a group of rats (n = 7) received infusions of 200 pmol cocaine/100 nl aCSF for 8 consecutive sessions.

Co-infusion of ICS 205,930 with 200 pmol/100 nl Cocaine

Rats were randomly assigned to one of three groups (n = 6/group). All groups self-administered 200 pmol/100 nl cocaine for the initial 4 sessions, 200 pmol/100 nl cocaine with 50, 100, or 200 μM ICS 205,930 during session 5 and 6, and 200 pmol/100 nl cocaine during session 7. In a previous study, we examined the effects of co-administration of three 5-HT_3 antagonist on the self-infusion of EtOH into the posterior VTA (Rodd-Henricks et al., 2003). Responses on the active and inactive lever for 100 μM zacopride, LY278-584, or ICS 205,930 were simlar to responses on these levers for aCSF self-infusions into the posterior VTA of Wistar rats (Rodd-Henricks et al., 2003), suggesting that these antagonists do not impair operant responding when given into the posterior VTA . Another ICSA study indicated that higher concentrations of ICS 205,930 (400 μM) did not affect acetaldehyde self-administration (> 100 active lever responses/session) into the posterior VTA (Rodd et al., 2004c). Furthermore, bilateral microinjections of ICS 205,930 (up to 1.5 μg/side) in alcohol-preferring (P) rats prior to operant oral self-administration of ethanol increased responding for ethanol by 120% when microinjected into either the posterior VTA or AcbSh (Pommer et al., 2004). Thus, the ICS alone control group was not included in the present

study because prior studies (see above) indicated that by itself ICS 205,930 did not impair lever responding.

Co-infusion of quinpirole with 200 pmol/100 nl Cocaine

To determine whether activation of DA neurons in the posterior VTA is required for cocaine self-administration, we examined the effects of co-administration of the $D_{2/3}$ agonist quinpirole on posterior VTA cocaine self-administration. The concentrations of quinpirole used were derived from studies indicating that co-administration of 1 μM did not alter ethanol or acetaldehyde self-administration into the posterior VTA, but 100 μM was effective at reducing self-administration (Rodd et al., 2004a, 2004b). All rats self-administered 200 pmol/100 nl cocaine for the initial 4 sessions, 200 pmol/100 nl cocaine with 1 μM quinpirole during session 5, 200 pmol/100 nl cocaine with 100 μM quinpirole during session 6, and 200 pmol/100 nl cocaine during session 7. Previous data indicated that rats given quinpirole alone to self-infuse into the posterior VTA had similar responding on the active and inactive levers as an aCSF control group (Rodd et al., 2004b, c). Thus, the quinpirole alone control group was not included in the present study because prior studies indicated that quinpirole alone does not alter operant responding.

Histology

At the termination of the experiment, 1% bromophenol blue (0.5 ul) was injected into the infusion site. Subsequently, the animals were given a fatal dose of Nembutal and then decapitated. Brains were removed and immediately frozen at -70^0 C. Frozen brains were equilibrated at -15^0 C in a cryostat microtome and then sliced into 40 um sections. Sections were then stained with cresyl violet and examined under a light microscope for verification of the injector site using the rat brain atlas of Paxinos and Watson (1986).

Statistical Analysis

Data analysis consisted of a group x day mixed ANOVA, with a repeated measure of 'day', preformed on the number of infusions. Additionally, for each individual group, lever discrimination was determined by type (active or inactive) x day mixed ANOVA with a repeated measure of 'day'. Lever discrimination is a key factor when a stimulant is self-administered (e.g., ethanol, cocaine, amphetamine) to distinguish between reinforcement-contingent behavior and drug-stimulated locomotor activity.

Results

The anterior VTA was defined as the VTA region at the level of the mammilary nuclei, 4.8 to 5.2 posterior to bregma (Fig. 1). The posterior VTA was defined as the VTA region at the level of the interpeduncular nucleus, coronal sections at –5.3 to –6.04 bregma (Fig. 1). Cannula placements surrounding the VTA included injection sites located in the substantia nigra, red nucleus, and caudal linear nucleus of the raphe. Rats with injector tip placements outside the VTA displayed an overall low level of infusions and active lever responding throughout all sessions (average infusions and active lever responses for initial 4 sessions – 6.0 $\pm$ 0.5 and 13.2 $\pm$ 0.8, respectively). For all sessions, the number of infusions of cocaine outside the VTA was not significantly different than the aCSF group with injection sites in the VTA (p values > 0.28). Similarly, examination of the active lever responses revealed that rats administering cocaine into areas outside the VTA displayed equivalent amounts of low levels of responding on both the active and inactive levers (p values > 0.41).

Dose Response

A select range of cocaine concentrations infused into the posterior VTA supported response-contingent behaviors. For rats receiving infusions into the posterior VTA, an ANOVA on the average number of infusions (Figure 2, left panel) received during the initial 4 test days (acquisition) revealed a significant effect of Group ($F_{6,46} = 18.5$; $p < .0001$). Post-hoc comparisons (Tukey's b) indicated that the 50, 100, and 200 pmol/100 nl cocaine groups received significantly more infusions than rats self-administering aCSF, 25 or 400 pmol/100 nl cocaine. Additionally, rats self-administering 100 and 200 pmol/100 nl infused more often than rats self-administering 50 pmol/100 nl. In contrast, in the anterior VTA none of the cocaine concentrations were significantly self-infused above aCSF levels (Fig. 2; $p = 0.52$).

The temporal patterns of infusions (in 30-min blocks) by rats given aCSF or 200 pmol/100 nl cocaine into the posterior VTA or 200 pmol/100 nl cocaine into the anterior VTA are depicted in Figure 3. Throughout all sessions, rats self-administering aCSF into the posterior VTA closely resembled the infusion pattern for rats that were self-administering 200 pmol cocaine into the anterior VTA. For the group self-infusing 200 pmol cocaine into the posterior VTA, approximately 90 min into the first session, this group diverged from the other two groups and displayed signs of acquiring self-administration behavior (Fig. 3, middle panel). During session 4, rats readily administered 200 pmol cocaine/100 nl within the first 30-min block, which gradually decreased during the next three 30-min blocks, and then gradually increased during the remaining four 30-min blocks. During the second aCSF substitution session (session 6), the number of infusions was low and most were administered during the initial two 30-min periods. When 200 pmol cocaine was restored in session 7, rats

rapidly reinstated self-administration behavior and exhibited an infusion pattern similar to session 4.

Throughout the sessions, responses on the active lever for the self-infusion of aCSF into the posterior VTA was low (20 or fewer responses/session) and did not differ with regard to responses on the inactive lever ($p = 0.87$; data not shown). The 50, 100, and 200 pmol/100 nl cocaine infusate groups (Fig. 4) responded significantly more on the active than inactive lever throughout the 4 acquisition sessions (p values < 0.001). When aCSF was substituted for cocaine in sessions 5 and 6, responses on the active lever decreased to the low levels observed for the inactive lever (p values > 0.17). When cocaine was restored in session 7, responding on the active lever increased and was significantly higher than responses on the inactive lever in rats self-administering 50, 100, or 200 pmol/100 nl cocaine (p values < 0.004). Rats given 25 or 400 pmol/100 nl cocaine had low levels of responding on the active lever (25 responses/session) which were not different than responses on the inactive lever (data not shown). In contrast to the posterior data, rats given the same concentrations of cocaine to self-infuse into the anterior VTA had low level of responding on the active lever (range 18.8 $\pm$ 5.3 to 23.4 $\pm$ 8.1 responses/session) that did not differ responses on the inactive lever (range 15.5 $\pm$ 9.9 to 21.6 $\pm$ 7.9 responses/session) or from active levers responses for the aCSF group (24.8 $\pm$ 8.2 responses/session).

Higher Cocaine Concentrations

Higher concentrations of cocaine were tested in a separate experiment and with a slightly different paradigm. Neither 800 nor 1600 pmol/100 nl cocaine was self-infused into either the posterior (15 $\pm$ 2 and 10 $\pm$ 3, respectively) or anterior (5 $\pm$ 2 and 4 $\pm$ 2, respectively) VTA. For rats with placements in the posterior VTA, there was no evidence for

lever discrimination (all p values > 0.33) and no alteration in self-administration across session (Session $F_{7,49} = 1.2$; $p = 0.32$). Similarly, for rats self-administering into the anterior VTA there was also no indication of lever discrimination (all p values > 0.41) or effect of session ($F_{7,49} = 0.76$; $p = 0.46$). The number of self-infusions observed in both areas for the 800 and 1600 pmol/100 nl concentrations of cocaine were equivalent to the infusions of aCSF self-administered into the corresponding brain area in the initial dose-response experiment.

Extended Time-Out

Rats given 200 pmol/100 nl cocaine to self-infuse under the 5-sec and 25-sec timeout conditions responded significantly more on the active than inactive lever throughout all 8 sessions (Fig. 5 top panel; p values < 0.001). Extending the timeout period between infusions during sessions 5-8 resulted in an almost 2-fold increase in responding on the active lever. The number of infusions received during sessions 5-8 did not differ from infusions received in sessions 3 and 4 (Fig. 5, bottom panel). Examining active lever responses from sessions 3-8, there was a significant effect of session ($F_{5,30} = 9.3$; $p < 0.000$), and post-hoc contrasts revealed that active lever responses were elevated (2-fold) during sessions 5-8 compared to sessions 3 and 4. The number of infusions received remained constant during this time period ($F_{5,30} = 1.6$; $p = 0.18$). A detailed examination of the time of each lever response indicated that the 'additional' active lever responses observed during the 25-sec timeout condition occurred around 10 sec after the infusion, approximately the time when the rats would be allowed to infuse under the 5-sec timeout condition.

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Co-infusion of ICS 205,930 with 200 pmol/100 nl Cocaine

Throughout the 4 acquisition sessions, rats readily self-infused 200 pmol cocaine/100 nl (25 $\pm$ 3 infusions/session) and responded significantly more on the active than inactive lever (all F values$_{1,5}$ > 12.9; all p values < 0.016; Fig. 6). Co-administration of 50 μM ICS 205,930 in sessions 5 and 6 (Fig. 6, top panel) did not significantly alter responding on the active lever or the number of self-infusions (all F values$_{2,10}$ < 0.9; all p values > 0.44). However, co-administration of 100 (Fig. 7, middle panel) or 200 μM (Fig. 6, bottom panel) ICS 205,930 in sessions 5 and 6 reduced the number of active lever responses and self-infusions (all F values$_{2,10}$ > 8.8; all p values < 0.006). The 200 μM concentration of ICS 205,930 was effective in both sessions, whereas the 100 μM concentration was effective only in session 6. When cocaine alone was given during session 7, responding on the active lever and the number of self-infusions increased to levels observed in session 4 (all F values$_{2,10}$ < 0.1; all p values > 0.90).

Co-infusion of quinpirole with 200 pmol/100 nl Cocaine

Throughout the 4 acquisition sessions, rats readily self-infused 200 pmol cocaine/100 nl (26 $\pm$ 5 infusions/session) and responded significantly more on the active than inactive lever (all F values$_{1,5}$ > 13.3; all p values < 0.015; Fig. 7). Co-administration of 1 μM quinpirole in session 5 (Fig. 7) did not significantly alter responding on the active lever or the number of self-infusions (all F values$_{1,5}$ < 0.2; all p values > 0.67). However, co-administration of 100 μM quinpirole during session 6 (Fig. 7) reduced the number of active lever responses and self-infusions compared to session 4 (all F values$_{1,5}$ > 9.3; all p values <

0.030). When cocaine alone was given during session 7, responding on the active lever and the number of self-infusions increased to levels observed in session 4 (all p values > 0.82).

Discussion

The results of this study indicate that Wistar rats will initiate and maintain the self-infusion of cocaine into the posterior, but not anterior, VTA (Figs. 2 and 3). Furthermore, the self-infusion of cocaine into the posterior VTA does not appear to be a result of a general increase in behavioral activity because Wistar rats readily learn to discriminate the active from the inactive lever for the self-infusion of 50-200 pmol/100 nl cocaine (Fig. 4). In addition, when aCSF was substituted for cocaine in sessions 5 and 6, responses on the active lever decreased to the low levels observed for the inactive lever, and responding on the active lever was reinstated when cocaine was restored. These findings, together with the pattern of infusions (Fig. 3) and the observation that the number of infusions was maintained with a 5-fold increase in the timeout duration (Fig. 5), provide support for an interpretation that cocaine is reinforcing within the posterior VTA of Wistar rats. The finding that co-administration of a 5-HT_3 antagonist reduced cocaine self-administration (Fig. 6) suggests that the reinforcing effects of cocaine in the posterior VTA are mediated in part through activation of local 5-HT_3 receptors. Additionally, the findings that co-administration of the $D_{2/3}$ agonist quinpirole reduced cocaine self-administration (Fig. 7) suggest that cocaine self-administration into the posterior VTA is dependent upon neuronal activation of VTA DA neurons.

The finding that the reinforcing effects of cocaine are heterogeneous within the VTA is compatible with other microinfusion studies. Arnt and Scheel-Kruger (1979) demonstrated

functional differences between the anterior and posterior VTA in the locomotor activating effects of $GABA_A$ agonists and antagonists. $GABA_A$ antagonists are self-infused into the anterior but not posterior VTA, while $GABA_A$ agonists are self-infused into the posterior but not anterior VTA (Ikemoto et al 1997a; 1998). Additionally, ethanol and acetaldehyde are self-administered into the posterior, but not anterior, VTA (Rodd-Henricks et al., 2000, 2002a; Rodd et al., 2004b, c). In mice, research has shown that injections of a $GABA_B$ agonist into the posterior, but not anterior, VTA increased locomotor activity (Boehm et al., 2002). The sub-regional differences in self-administration of cocaine are likely related to the differences in neuronal circuitry between the anterior and posterior VTA.

There is also regional heterogeneity within the VTA in response to opioids. Immunohistochemical studies have indicated that the posterior VTA has a greater density of μ–opioid receptors in the posterior than the anterior VTA (Mansour et al., 1995). Microinjections of endomorphin-1 (EM-1), a selective μ–opioid receptors agonist, were more effective at developing self-administration behaviors in the posterior than anterior VTA, and EM-1 conditioned a place preference and stimulated locomotor activity when injected into the posterior, but not anterior, VTA (Zangen et al., 2002). An intracranial conditioned place preference study indicated that the VTA differentially supported opioid reinforcement (Bozarth, 1987). Over-expression of an AMPA receptor subunit (GluR1) in the posterior and anterior VTA produced differential consequences on morphine conditioned place preference (Carlezon et al., 2000).

The findings that a $5\text{-}HT_3$ antagonist (ICS 205,930) can block the reinforcing effects of cocaine within the posterior VTA (Fig. 6) and that there is high 5-HT innervation of the posterior VTA compared to the anterior VTA (Herve et al., 1987) support an involvement of

the 5-HT system, and 5-HT_3 receptors in mediating the reinforcing properties of cocaine within the posterior VTA. Research findings have shown that cocaine can act at the 5-HT_3 receptor (Brietinger et al., 2001), and chronic cocaine treatment has been associated with a functional down-regulation of the 5-HT_3 receptor (King et al., 1995, 2002; Matell and King, 1997). Furthermore, mice that over-express the 5-HT_3 receptor are more sensitive to the locomotor activating effect of cocaine, but less sensitive to the reinforcing effects of cocaine in conditioned place preference (Allan et al., 2001). Systemically administered 5-HT_3 antagonists reduce cocaine-induced DA release in the Acb, and decrease cocaine-induced condition place preference and locomotor activity (Kankaanpaa et al., 2002). Therefore, the current findings extend previous research indicating the ability of the 5-HT_3 receptor to modulate the effects of cocaine.

Cocaine is a general monoamine reuptake inhibitor for DA, 5-HT, and norepinephrine (NE) neurons, and can stimulate the release of glutamate and other excitatory amino acids (Cornish and Kalivas, 2001). The relative affinity of cocaine to block monoamine transporters is disputable with some laboratories reporting 10-40 times greater affinity for the 5-HT transporter than the DA transporter (c.f. King et al., 2002), and other laboratories indicating an at least equimolar affinity of cocaine for the DA and 5-HT transporter (c.f. Cornish and Kalivas, 2001; Chen and Reith, 1994). Within the VTA, a reverse microdialysis study reported that low concentrations of cocaine (1-10 uM) dramatically increased the extracellular levels of 5-HT, NE, and DA equally (Chen and Reith, 1994). However, the levels of DA observed during the perfusion of the low concentrations of cocaine could be the result of 5-HT and NE stimulated activity of VTA DA neurons, which increased the extracellular levels of DA, and not solely from cocaine effects on the DA transporter (Chen

and Reith, 1994). In the present study, cocaine is being self-infused into the region of the VTA (Figs 2-4) with the highest 5-HT innervation (Herve et al., 1987). Local activation of 5-HT_3 receptors stimulates somatodendritic DA release in the VTA (Campbell et al., 1996) and bath application of cocaine increases VTA DA neuronal activity in midbrain tissue slices (Bunney et al., 2001). Therefore, it is possible that the reinforcing effects of cocaine within the posterior VTA may be mediated in part by blocking the reuptake of 5-HT at 5-HT_3 and other 5-HT receptor sites. Self-infusion of cocaine into the posterior VTA shows an apparent inverted 'U-shaped' dose-response curve (Fig. 2). The reduction in self-infusions at the higher concentrations may be a result of blocking DA reuptake which would then increase the extracellular concentrations of DA thereby activating D_2 autoreceptors and decreasing the firing rate of VTA DA neurons. The finding that co-administration with the $D_{2/3}$ agonist quinpirole reduced self-administration of cocaine suggests that activation of DA neurons is required for the self-infusion of cocaine into the posterior VTA (Fig. 7). Local administration of quinpirole will activate cell body D_2 autoreceptors and reduce DA neuronal activity, thereby preventing cocaine activation (Congar et al., 2002; Jeziorski and White 1989).

Another recent study has indicated that the VTA is a neuroanatomical site mediating the reinforcing effects of cocaine (David et al., 2004). Mice will learn to discriminate between two arms of a Y-maze to obtain microinjections of cocaine into the VTA at concentrations within the range that supported self-infusion in the current study. Systemic administration of either a D_1 or 5-HT_{1B} antagonist inhibited the discrimination between the two arms (David et al., 2004). An intriguing aspect of the David report was that the authors specifically targeted the posterior VTA.

The sensitivity of the posterior VTA to support cocaine self-administration is 16-fold greater than that of the AcbSh (Rodd-Henricks et al., 2002b). Under similar testing conditions, Wistar rats reliably self-administered cocaine into the AcbSh at concentrations of 800-1600 pmol/100 nl (8 to 12 mM; Rodd-Henricks et al., 2002b). Within the posterior VTA, cocaine can induce response-contingent behaviors between 50-200 pmol/100 nl (0.5 to 2 mM). Cocaine self-administration in the mPFC was reported to occur at concentrations ranging from 0.5 to 0.9 mM (Goeders and Smith, 1983), which are within the range reported here (Fig. 2).

Previous research attempting to determine if cocaine would be self-administered into the VTA varies slightly from the current study. In the initial study (Goeders and Smith, 1983), it was reported that concentrations between 0 and 5000 pmol/100 nl of cocaine failed to support self-administration in the VTA. However, from the histological presentation of cannulae placements, it would appear that most of the placements from that study were located in the anterior VTA, an area that did not support self-administration of cocaine in the current study. Yet, there are a number of methodological distinctions, besides injector location, between the current study and the reports of Goeders and Smith. Goeders and Smith's experiments used male Fisher 344 rats (Goeders and Smith, 1983, 1986, 1993; Goeders et al., 1986) while the current experiment used female Wistar rats. Fisher 344 rats are not as responsive to cocaine as many other rat lines, and are less likely to acquire i.v. self-administration of various drugs of abuse, including cocaine (Kosten et al., 1994, 1997; Ambrosio et al., 1995). Thus, injector location, the use of a different rat line, different gender, and other experimental parameters may have contributed to different findings regarding the self-administration of cocaine into the VTA.

Overall, the present data suggest that the posterior VTA is a neurobiological substrate for the rewarding effects of cocaine. Furthermore, the present data indicate that the reinforcing properties of cocaine in the posterior VTA are mediated through activation of DA neurons and local 5-HT_3 receptors.

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FIGURE LEGENDS

Fig. 1 Illustration depicts the injection sites in the anterior and posterior VTA, and sites outside of the posterior VTA, of female Wistar rats self-administering aCSF or various concentrations of cocaine. Closed circles represent placements of injection sites within the posterior VTA (defined as –5.4 to –6.0 mm Bregma), closed squares represent placements of injection sites in the anterior VTA (defined as –4.8 to –5.3 mm Bregma), and open squares represent injection placements outside the posterior VTA.

Fig. 2 The average number of infusions (± SEM) across the initial 4 sessions (acquisition) as a function of infusate concentration (0 to 400 pmol/100 nl; n = 6-10/group) and cannula placement (posterior or anterior VTA). *Asterisk* indicates infusions significantly higher than aCSF and 25 pmol/100 nl cocaine infusions; *plus* symbols indicate significantly higher values compared to aCSF, and 25 and 400 p mol/100 nl cocaine ($p < 0.05$; Tukey's b post-hoc).

Fig. 3 Representative infusion patterns records for Wistar rats self-administering aCSF into the posterior VTA or 200 pmol/100 nl cocaine into the anterior or posterior VTA in 30-min blocks (mean ± SEM). The top panels represent the initial acquisition session. The 2nd row of panels shows the 4th acquisition session. The 3rd row of panels shows the 2nd extinction session (session 6). The bottom row of panels shows the reinstatement session (7th overall session).

Fig. 4 The number of active and inactive lever presses (means ± SEM; n = 6-10/group) for rats self-administering 50, 100, or 200 pmol/100 nl cocaine into the posterior VTA during sessions 1-4, aCSF for sessions 5 and 6, and original infusate during session 7. *Asterisks* represent significant ($p<0.05$; Tukey's) difference from responding observed for rats self-administering aCSF and lever discrimination ($p < 0.05$) within a given infusate group (determined by one-way ANOVAs performed on individual sessions contrasting active and inactive lever presses).

Fig. 5 Number of active and inactive lever presses (means ± SEM; n = 7) for rats self-administering 200 pmol/100 nl cocaine into the posterior VTA under 5- or 25-sec infusion timeout periods. *Asterisks* represent significant lever discrimination ($p < 0.05$) within a given session (determined by one-way ANOVAs performed on individual sessions contrasting active and inactive lever presses). *Pound Symbols* represent elevated levels of responding compared to session 3 and 4. There were no significant differences in the number of infusions among the sessions.

Fig. 6 Effects of ICS 205,930 on responding for the self-infusion of 200 pmol/100 nl cocaine into the posterior VTA of female Wistar rats. For the first 4 sessions, 200 pmol/100 nl cocaine alone was given. In sessions 5 and 6, ICS 205, 930 (50, 100 or 200 μM) was co-infused with 200 pmol/100 nl cocaine. In session 7, only 200 pmol/100 nl cocaine was given. Date are the means ± S.E.M; n = 6/group. *$p < 0.05$ responses on the active lever significantly higher than responses on the inactive lever for that session.

Fig. 7 Effects of quinpirole on responding for the self-infusion of 200 pmol/100 nl cocaine into the posterior VTA of female Wistar rats. For the first 4 sessions, 200 pmol/100 nl cocaine alone was given. In sessions 5, 1 μM quinpirole was co-infused with 200 pmol/100 nl cocaine. During session 6, 100 μM quinpirole was co-infused with 200 pmol/100 nl cocaine. In session 7, only 200 pmol/100 nl cocaine was given. Date are the means $\pm$ S.E.M; n = 8. *$p < 0.05$ responses on the active lever significantly higher than responses on the inactive lever for that session. + $p < 0.05$ number of infusions significantly lower than average infusions in sessions 3 and 4.

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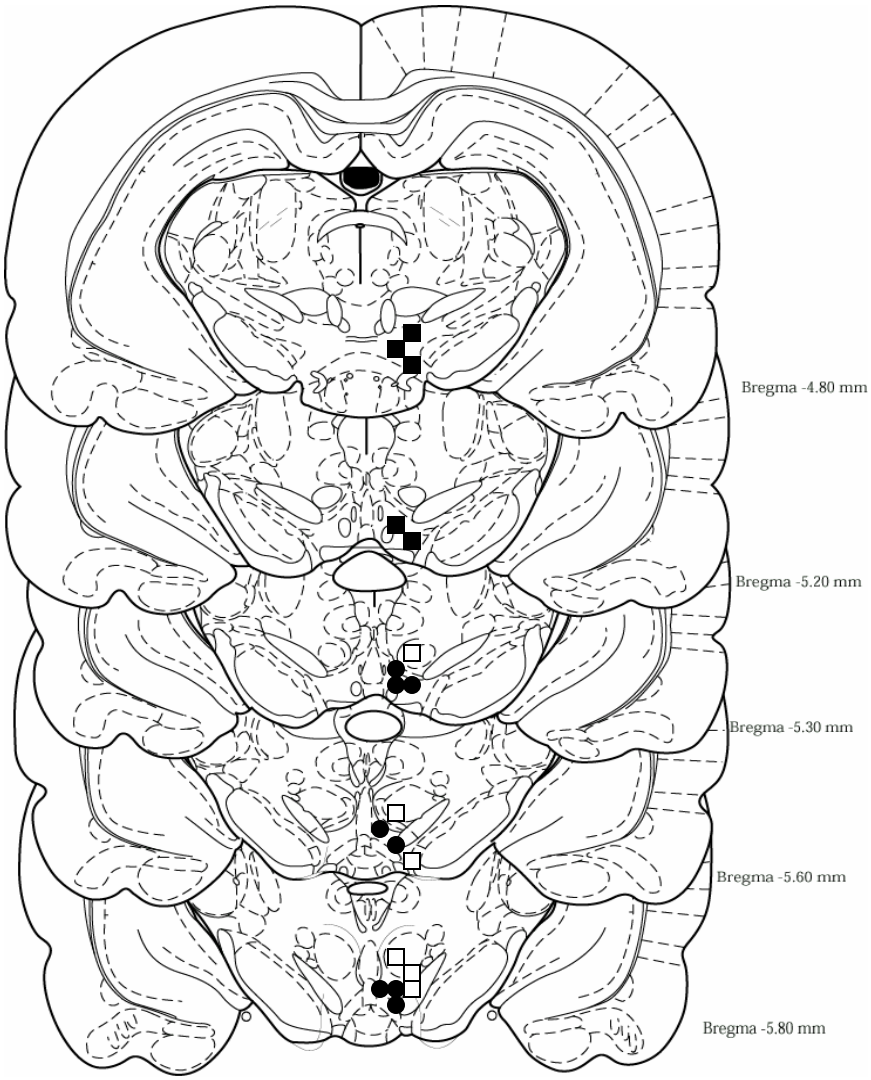

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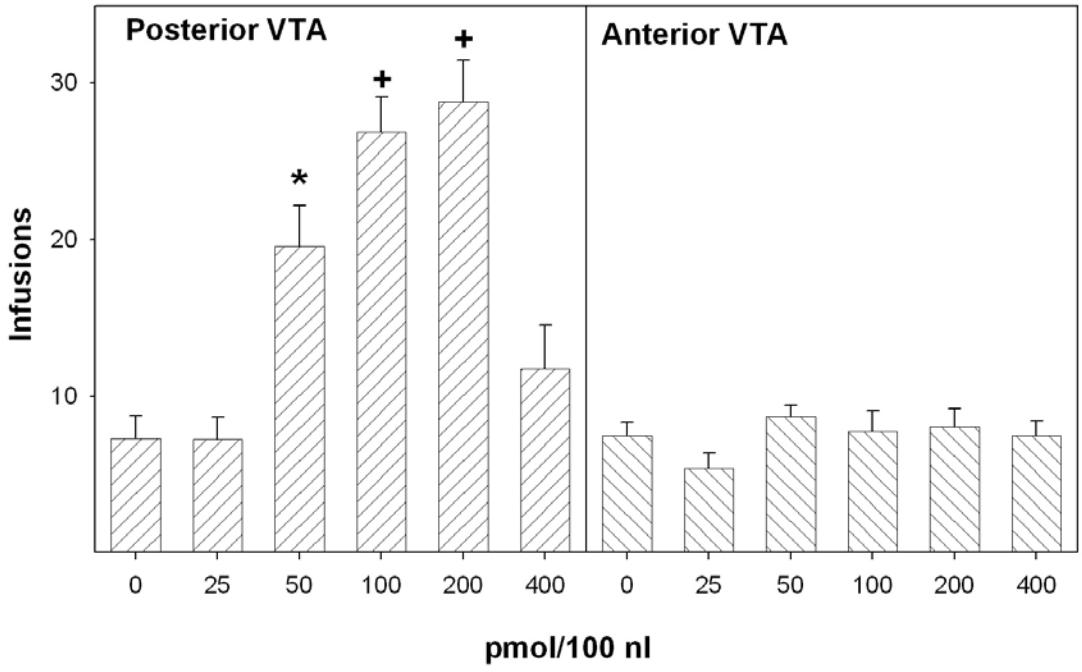

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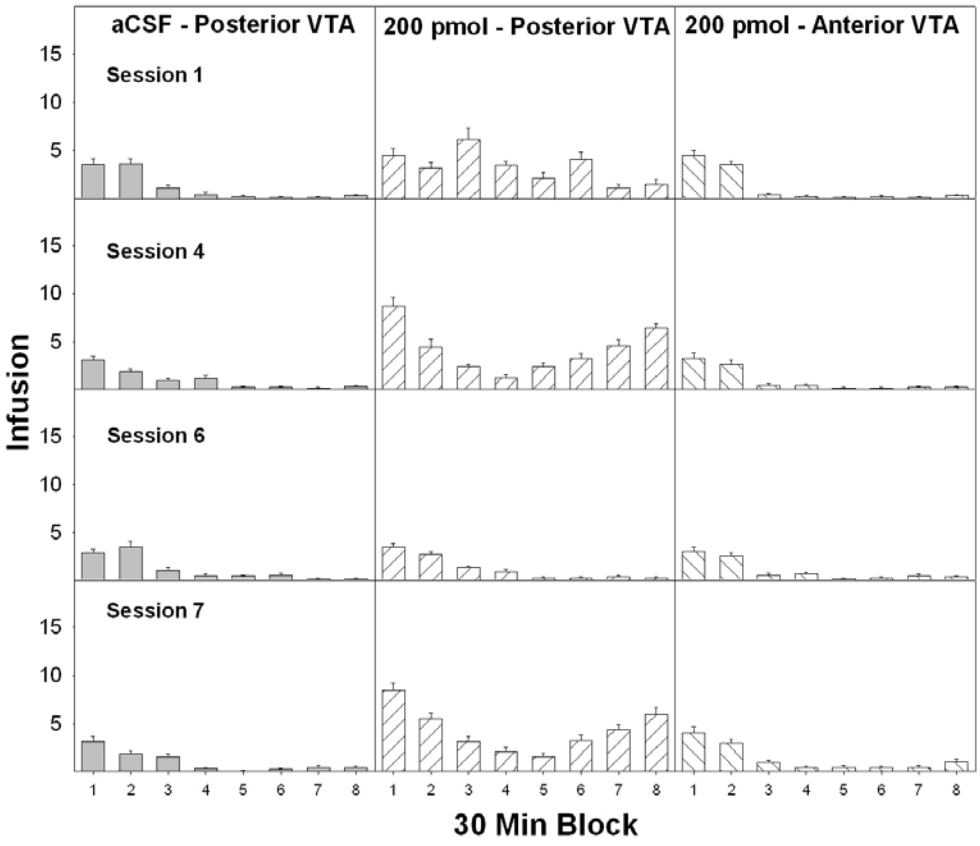

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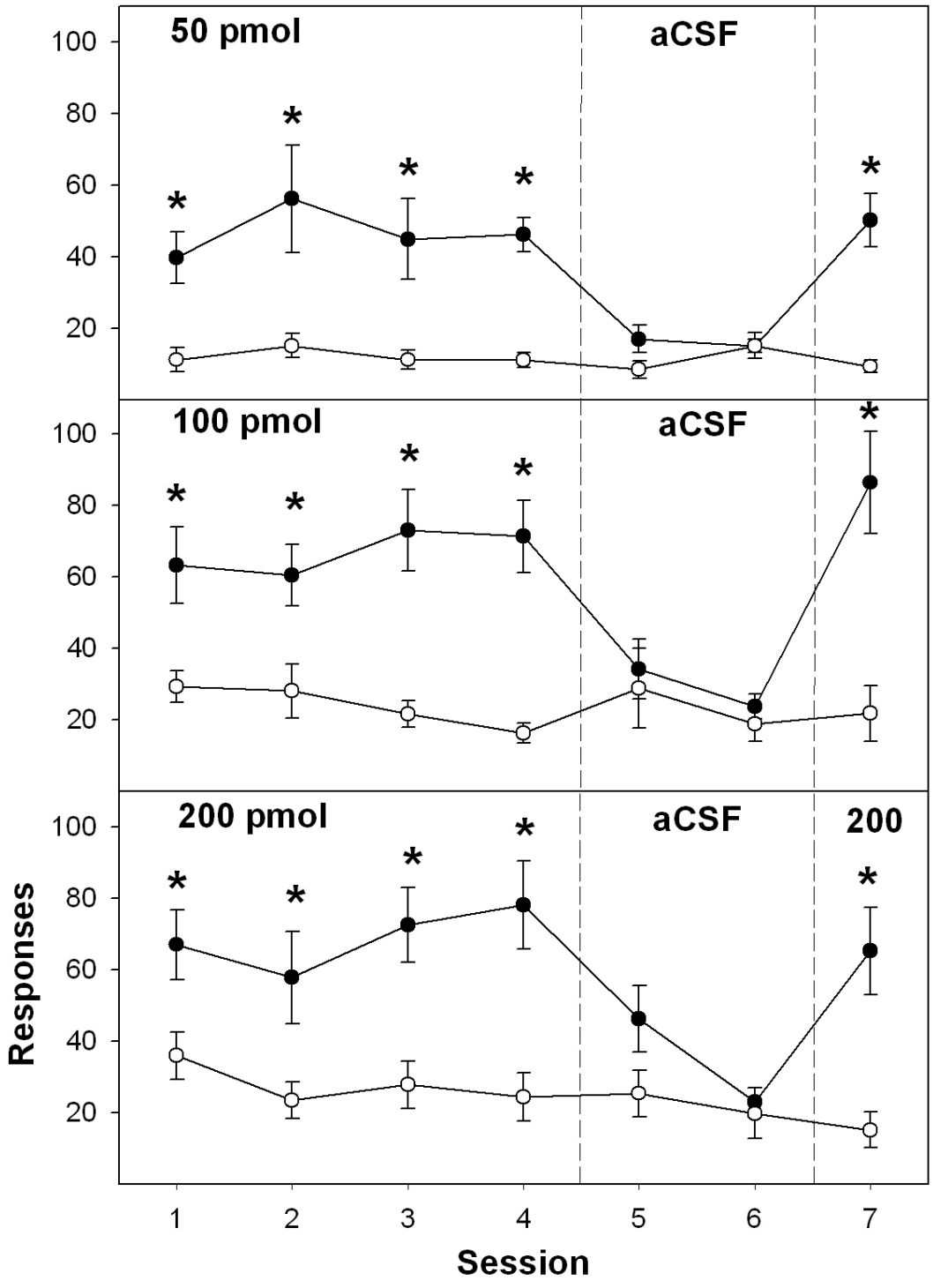

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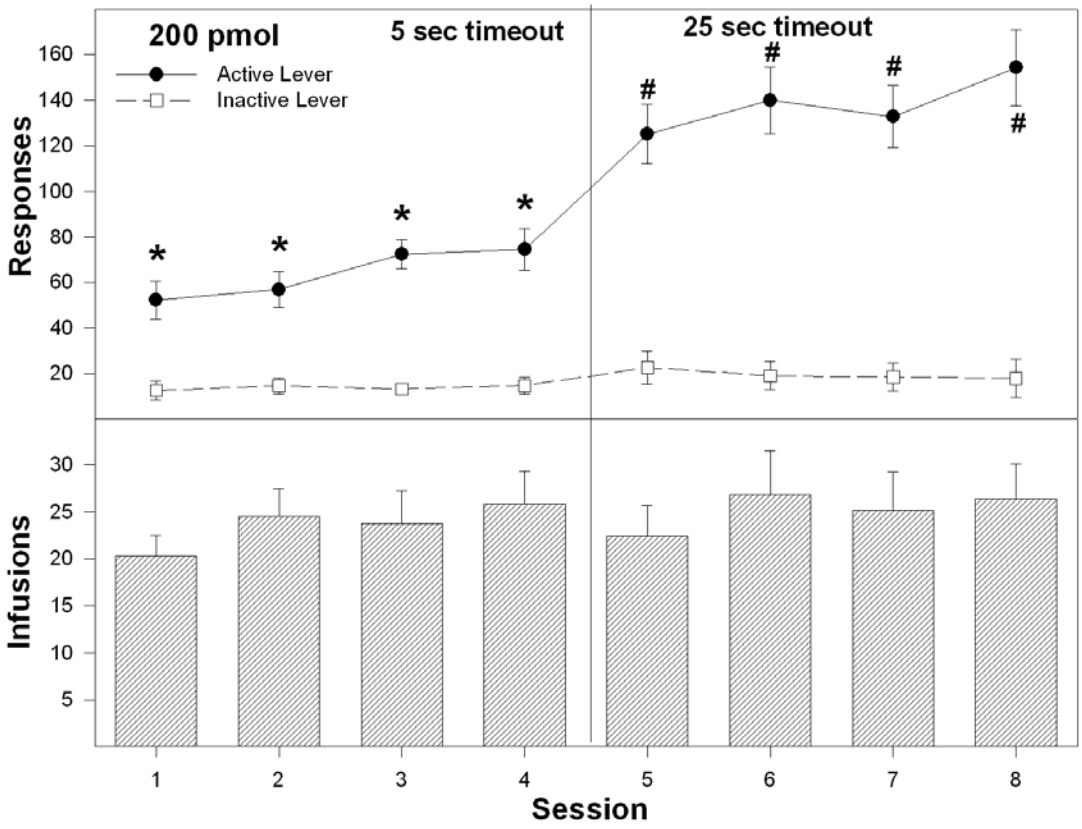

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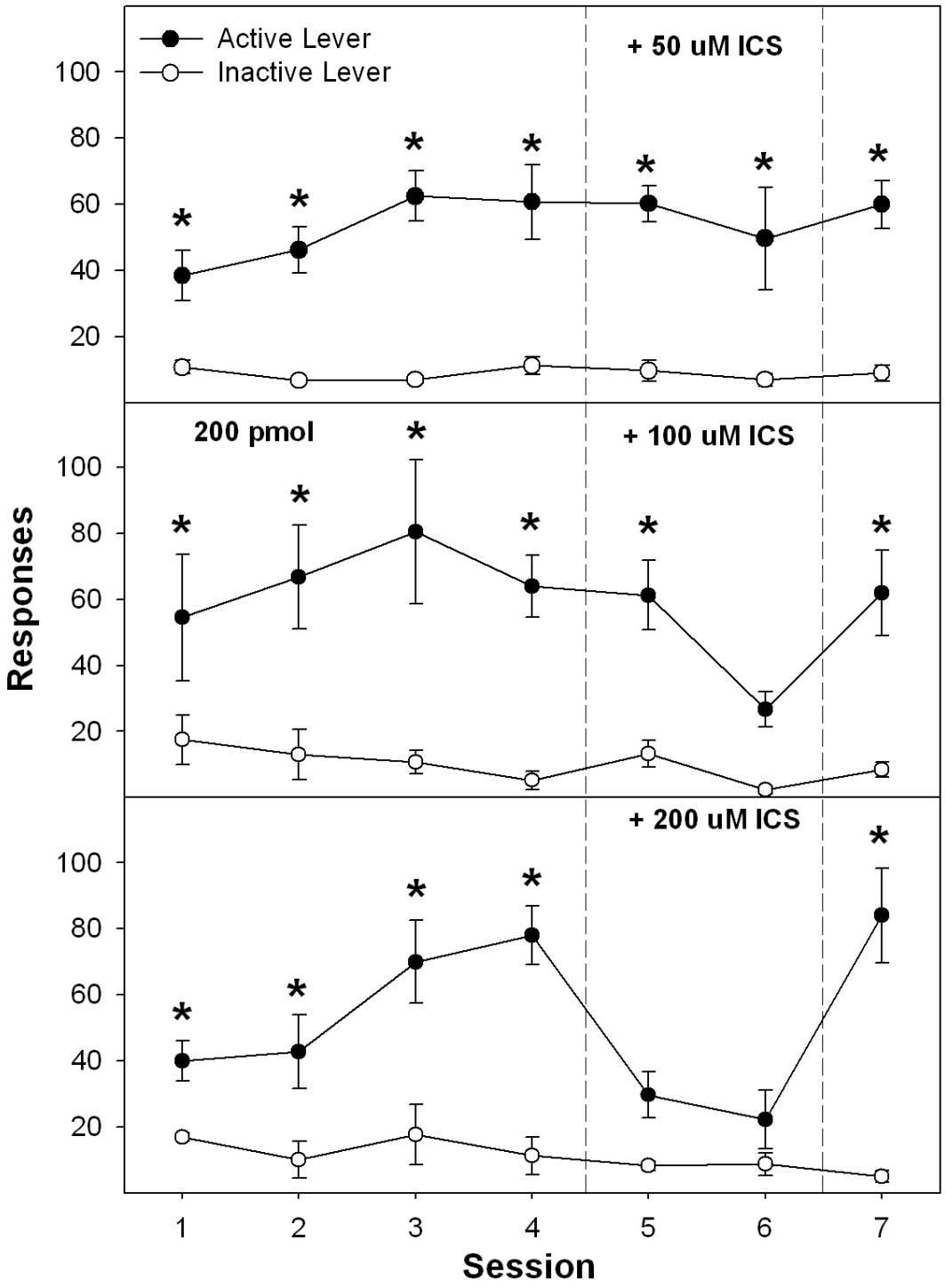

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