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Brain Research 651 (1994) 283–292

BRAIN RESEARCH

Research Report

The effects of systemic and intracerebral injections of D_1 and D_2 agonists on brain stimulation reward

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Accepted 5 April 1994

Abstract

That dopamine (DA) plays a role in reward-related learning is well documented but the mechanisms through which it acts are not well understood. The present set of experiments investigated the role of DA receptor subtypes within DA-innervated forebrain regions in brain stimulation reward (BSR). Thirty-two rats were implanted with electrodes in the ventral tegmental area (VTA) and cannulae aimed at the caudal nucleus accumbens (NAcc), the caudate-putamen (CP) or cortex. Rate–frequency functions were determined by logarithmically decreasing the number of cathodal pulses in a stimulation train from a value that sustained maximal responding to one that did not sustain responding (thresholds). After BSR thresholds stabilized rats received treatments with DA agonists and their effects on thresholds were analyzed. Systemic treatments consisted of injections of (+)-amphetamine (1.0 mg/kg, i.p., 10 min before testing), the D_2 agonist quinpirole (1.0 mg/kg, i.p., 10 min before testing), the novel D_1 agonist A-77636 (3.0 mg/kg, s.c., 90 min before testing) or their vehicle (distilled H_20). Central treatments consisted of microinjections of quinpirole (0.3–10.0 μg/0.5 μl) directly into the caudal NAcc, CP or cortex or A-77636 (30 μg/0.5 μl) into the caudal NAcc or CP. Results showed that all three agonists, when injected systemically, significantly reduced the threshold frequency required for VTA BSR, indicating a potentiative effect on reward. Central injections of quinpirole in the caudal NAcc, CP or cortex produced significant increases in BSR thresholds indicative of reduced rewarding efficacy of stimulation. Central injections of A-77636 into the caudal NAcc, but not the CP, were associated with a reduction in VTA BSR thresholds, suggesting an increase in reward. These results suggest that stimulation of D_1 or D_2 receptors enhances the rewarding effect of brain stimulation. In the case of the systemic quinpirole enhancement of reward, the present results suggest that this may not occur in the caudal NAcc, CP or cortex. Finally, the present results suggest that D_1 receptor stimulation in the caudal NAcc can facilitate reward-related learning.

Key words: A-77636; Amphetamine; Brain stimulation reward; Caudate-putamen; Cortex; D_1 agonist; D_2 agonist; Dopamine receptor; Nucleus accumbens; Quinpirole; Rat; Reinforcement

1. Introduction

There now exists considerable psychopharmacological evidence that dopamine (DA) plays an important role in reward-related learning. When DA antagonists were administered to animals performing operant responses for reward these drugs produced a reduction in the rewarding effects of the stimuli that controlled responding (for detailed reviews see [3,53,54]). Accordingly, when DA agonists were administered to animals the drugs tended to potentiate the rewarding effects of the controlling stimuli [6,14,16,19,20,43–45].

Anatomical studies have identified several DA-innervated regions within the mesotelencephalic system as being importantly involved in reward-related learning. Thus, it appears that responding controlled by food reward is dependent on intact DA transmission within the caudate-putamen (CP) [5,7,13,40] but not necessarily within the nucleus accumbens (NAcc) [1,13,46]. On the other hand, intact DA functioning in the NAcc has been shown to be involved in responding that is motivated by conditioned reward [8,24,49,50] and intravenous cocaine or opiate infusions [12,46] as well as in producing conditioned place preferences [11,20]. Hence, there is strong evidence that DA pro-

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SSDI 0006-8993(94)00458-0

jections from the ventral tegmental area (VTA) to the NAcc are involved in the ability of rewarding stimuli to control behavior.

The brain stimulation reward (BSR) paradigm has been used extensively to study the neuroanatomical and neurochemical substrates of reward [30,54] and the mesoaccumbens DA projections have been implicated. Thus, injections of DA antagonists into the NAcc reduced the rewarding effects of lateral hypothalamic self-stimulation [48] while injections of a DA agonist enhanced it [9]. These results corroborate studies that have looked at other rewards and are congruent with the suggestion that DA release in the NAcc, likely through activation of the mesoaccumbens system by BSR, may produce rewarding effects similar to those produced by cocaine, opiates and conditioned reward.

DA is believed to play a role in reward through its actions at post-synaptic receptors. DA receptors can be divided into at least five subtypes based on their molecular configuration [18,35,47] and are referred to as D_1 through D_5. However, these receptor subtypes can also be separated into two broader categories based on their relationship to the enzyme adenylate cyclase. In this categorization D_1 receptors (D_1 and D_5) are those that stimulate adenylate cyclase activity whereas D_2 receptors (D_2, D_3 and D_4) are those that either do not stimulate or inhibit adenylate cyclase activity [23]. In recent years pharmacological agents that act specifically on the D_1 or D_2 receptor families have been developed. These agents have been used as tools by researchers interested in the roles played by the specific DA receptor families in reward-related learning.

Several studies have investigated the role of DA receptor subtypes in BSR. Thus, systemic administration of the D_1 and D_2 receptor-specific antagonists, SCH 23390 and raclopride, respectively, reduced the rewarding effects of BSR [33,34]. Accordingly, systemic administration of the D_2 agonists, quinpirole and CV 205–502, increased the rewarding effects of BSR. The D_1-specific agonist, SKF 38393, failed to produce potentiative effects on BSR [34], a result that is congruent with those obtained in studies looking at other types of reward [6,56].

We know of only one study that has been aimed at investigating the role of DA receptor subtypes within the NAcc in BSR. In this study microinjections of SCH 23390 into the NAcc ipsilateral, but not contralateral, to the stimulation electrode reduced the rewarding efficacy of VTA stimulation [26]. To date there have been no studies investigating the effects of DA receptor-specific agonists in the NAcc on BSR. The present study was designed to investigate such effects.

It has been suggested that reward-related learning may consist of a DA signal at D_1 receptors [4,29]. This would lead to the prediction that the administration of a D_1 agonist would mask the putative reward signal and impair reward-related learning. This hypothesis finds support from studies showing that SKF 38393 impaired responding for conditioned reward [6] and failed to be self-administered [56]. The present study investigated the effects of a D_1 agonist on self-stimulation of the VTA. If responding for VTA rewarding stimulation is dependent on a D_1 signal then a D_1 agonist would be expected to increase self-stimulation thresholds.

The failure to observe potentiative effects on reward with systemic administrations of the D_1 agonist, SKF 38393 [6,34,56], may be due to its being a partial D_1 agonist [2,36]. We investigated the effects of a novel D_1 receptor agent, A-77636, that has full agonist properties [22] on reward-related learning.

Rats were implanted with electrodes aimed at the VTA and trained to self-stimulate. These rats then received systemic as well as intra-caudal NAcc injections of the full D_1 agonist, A-77636, and, for comparison, the D_2 agonist, quinpirole. The effects of these drugs on self-stimulation thresholds were evaluated.

2. Materials and method

Treatment of the rats in the present study was in accordance with the Animals for Research Act, the Guidelines of the Canadian Council on Animal Care and relevant University policy and was approved by the Queen's University Animal Care Committee.

2.1. Subjects

Subjects consisted of 32 male Wistar rats (Charles River Canada) weighing between 275 and 325 g at the time of surgery (approximately 5–7 days after arrival). The rats were housed in individual hanging wire cages and maintained on a 12 h light/dark cycle (lights on at 07.00 h) in a temperature-controlled environment (21°C). Purina rat chow and water were available to the rats ad libitum.

2.2. Apparatus

The experimental environments consisted of four similar operant chambers (29×23×18 cm high) constructed of aluminum sides and plexiglass backs, tops and doors. The top of each chamber contained a hole 3 cm in diameter for the stimulation lead. The floors were made of aluminum grids. Each chamber was placed in a ventilated sound-attenuating box. One of the 29 cm walls contained two 3.5×2.0 cm levers placed 8 cm apart. A force of 0.09 N was required to depress each lever. Only one lever was connected to the stimulator and pulse counter. A 2-W light bulb was situated 10 cm above each lever.

Experimental parameters (e.g. trial length) were controlled by one experimenter controller board [51] for each chamber using custom written software (Steve Ferguson, Queen's University).

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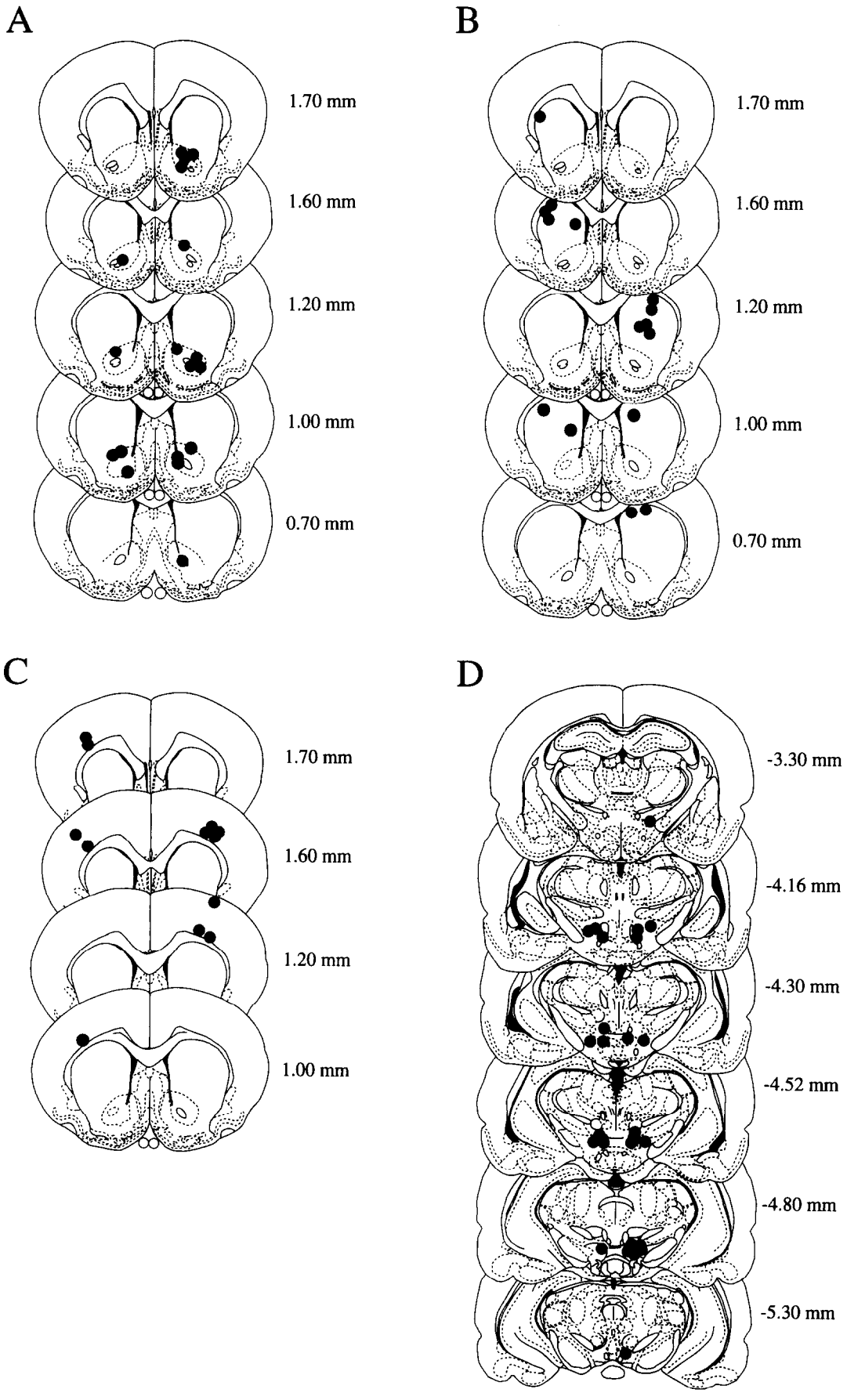
Fig. 1. Coronal sections showing the location of the (A–C) injector tips and (D) stimulating electrodes for all rats. Drawings are adapted from Paxinos and Watson [37]; the numbers beside each section indicate the distance (mm) anterior to bregma.


A
1.70 mm
1.60 mm
1.20 mm
1.00 mm
0.70 mm
B
1.70 mm
1.60 mm
1.20 mm
1.00 mm
0.70 mm
C
1.70 mm
1.60 mm
1.20 mm
1.00 mm
D
-3.30 mm
-4.16 mm
-4.30 mm
-4.52 mm
-4.80 mm
-5.30 mm

2.3. Surgical procedure

The rats were anaesthetized with sodium pentobarbital at 65 mg/kg b.wt. and fitted into the stereotaxic apparatus. Moveable electrodes [28] were aimed at the VTA using the stereotaxic coordinates of 4.8 mm posterior to bregma, 2.5 mm lateral from the midline at a 10° angle and 8.5 mm below the surface of the skull. Cannula guides were aimed at the caudal NAcc, the CP or the cortex using the following coordinates anterior to bregma, lateral to the midline and ventral to the surface of the skull: 0.8, 3.4 and 7.2 for the caudal NAcc; 0.8, 3.4 and 5.2 mm, for the CP and 0.8, 3.4 and 2.4 mm for the cortex. All guide cannulae were implanted at a 15° angle. The incisor bar was positioned 3.5 mm below the horizontal plane passing through the interaural line [37].

Electrodes consisted of a plastic guide and a moveable stainless steel wire (0.25 mm diam.) coated with epoxylite, except for the tip. The cannula guides (0.64 mm diam.) were made from modified 23 gauge stainless steel needles. The injectors (0.32 mm diam.) were made from 30 gauge stainless steel tubing. A 30 gauge stainless steel wire (0.32 mm diam.) was kept in the guide cannula between injections. Some rats received microinjections in two or three sites (cortex, CP and caudal NAcc). This was accomplished by appropriately varying the length of the injector. In such cases all dorsal injections were performed *before* the ventral ones and never vice versa.

2.4. Procedure

Following more than 1 week of post-operative recovery the rats were tested for self-stimulation using 0.3 s trains of cathodal rectangular pulses each lasting 0.1 ms. During the shaping period the current intensity and frequency were varied manually and finally fixed at parameters that sustained bar pressing. The rats then were allowed to press the lever freely for 1 h per day for 4 consecutive days. After the animals learned to press the lever for pulses, BSR thresholds were determined by setting the frequency of pulses at the value that sustained maximal responding and decreasing the frequency in decrements of approximately 0.055 log units of pulses until the rats stopped responding. Stimulation was available to the animals on a fixed interval schedule of 0.5 s (FI 0.5) for trials of 50 s with an intertrial interval of 15 s. The onset and offset of the lights signified the start and end of a trial, respectively. Presses on the rewarded lever were counted.

The testing period began when BSR thresholds were stable for each rat. A stable threshold was operationally defined as 3 consecutive sessions during which the threshold (calculated using the Gompertz sigmoidal model, see [10]) did not deviate from the mean threshold by more than 10%.

The testing period consisted of individual test sessions each separated by at least 48 h. Each rat was tested with 1–5 microinjections in any particular site and 2 systemic injections. The drugs used were (+)-amphetamine, quinpirole, A-77636, raclopride and SCH 23390, and each rat received the drugs in a different order. Only the systemic and central vehicle (distilled water), quinpirole and A-77636 and systemic amphetamine data are reported here. Test sessions began with 4 new determinations of BSR threshold after which the rats were removed from the operant chambers. Rats were fitted with an injection cannula connected to a 10-μl Hamilton syringe through a length of polyethylene tubing. Using an infusion pump (Sage Instruments) injections (0.5 μl) were delivered over a 30 s period. The injector was left in place for an additional 60 s to ensure diffusion of the drug. The central injections consisted of either distilled water (0.5 μl), quinpirole or A-77636. The systemic injections consisted of amphetamine (i.p., 10 min before testing), quinpirole (i.p., 10 min before testing) and A-77636 (s.c., 90 min before testing). In all conditions the rats were returned to the operant chamber and new determinations of self-stimulation thresholds were obtained for 30 min (90 min in the case of systemic A-77636).

2.5. Drug preparation

(+)-Amphetamine sulphate (Smith, Kline and French Canada Ltd.) was dissolved in 0.9% saline and injected in a concentration of 1.0 mg/kg b.wt. Quinpirole (Eli Lilly and Co.) and A-77636 (Abbott Laboratories) were dissolved in distilled water. Quinpirole was injected in concentrations of 1.0 mg/kg b.wt. systemically and 0.3, 1.0, 3.0 and 10 μg/0.5 μl centrally. A-77636 was injected in concentrations of 3.0 mg/kg b.wt. systemically and 30.0 μg/0.5 μl centrally. All drugs were prepared daily immediately prior to injection.

2.6. Histology

When the testing period was over the rats were injected with a lethal dose of sodium pentobarbital, exsanguinated with saline, perfused with formalin and decapitated. The brains were removed and placed in 10% formalin for 4–7 days. The brains then were cut in 60 μm serial sections and stained with thionin for verification of electrode and injector implantation sites. Placements were evaluated by a researcher who was blind to the results of behavioral testing.

2.7. Data analysis

Data gathered from the pre- and post-injection portions of each session were curve-fitted and threshold and asymptote estimates were obtained using the Gompertz sigmoidal growth model [10]. The post-injection threshold value was divided by the pre-injection threshold value to obtain a ratio. This calculation was performed for all conditions. Data from groups receiving systemic treatments were analyzed individually with a one-way analysis of variance (ANOVA) with repeated measures. Data from the groups receiving quinpirole within a particular site were analyzed individually using a 2-way ANOVA with independent groups (quinpirole dose) and repeated measures on the treatment factor (vehicle vs. quinpirole). Data from the groups receiving A-77636 in a particular site were also analyzed individually using a one-way ANOVA with repeated measures. A significant treatment effect would indicate a potentiation or reduction of VTA BSR (depending on the direction of change in the means) produced by the drug. A significant treatment by group interaction would indicate that the drug-produced change in BSR threshold depended on the dose of drug given. Although most rats received more than one drug or dose of drug injection, all of the statistical analyses used two measurements (vehicle and drug) for each animal (except for the cortex quinpirole analysis where data from 2 of the 15 rats appeared in two groups).

3. Results

Fig. 1 illustrates the location of the injector tips within the caudal NAcc, CP and cortex and the location of the stimulating electrodes for all rats. The histological analysis revealed 18 placements within the caudal NAcc, 15 placements within the CP and 12 placements within the cortex. All electrodes were found to be located in or just anterior to the VTA (because the brains of two rats were not retrieved, their electrode locations were not verified).

Fig. 2 depicts representative rate–frequency functions obtained before and after systemic injections of the DA agonists and the vehicle. Fig. 2A shows that the post-vehicle rate–frequency function was shifted very slightly to the right of the baseline. This effect was

observed to a similar degree in most of the rats receiving systemic or central injections of vehicle. Fig. 2 shows that each of the DA agonists produced parallel shifts to the left of the rate–frequency functions, indicating that frequencies which previously did not sustain responding did so after treatment with the DA agonists. The data also indicate that asymptotic rates of responding did not appear to be affected after systemic injections of amphetamine or A-77636 but were greatly reduced after systemic quinpirole.

Fig. 3A illustrates the mean ratios of pre-injection BSR thresholds obtained after systemic administration of (+)-amphetamine, quinpirole and A-77636. As expected, the mean ratios of pre-injection thresholds obtained after amphetamine and quinpirole were much smaller than those obtained after their vehicles. Moreover, the results showed that A-77636, at a dose of 3.0 mg/kg, produced mean ratios of pre-injection thresholds smaller than those produced by the vehicle injections and almost as low as those produced by amphetamine and quinpirole. Thus, the results appeared to indicate that each of the three drugs produced reward-enhancing effects.

Fig. 3B shows the mean ratios of pre-injection asymptotes for the three drug groups. The results demonstrate a slight enhancement of asymptotic responding after amphetamine administration compared to its vehicle, and a slight reduction after A-77636

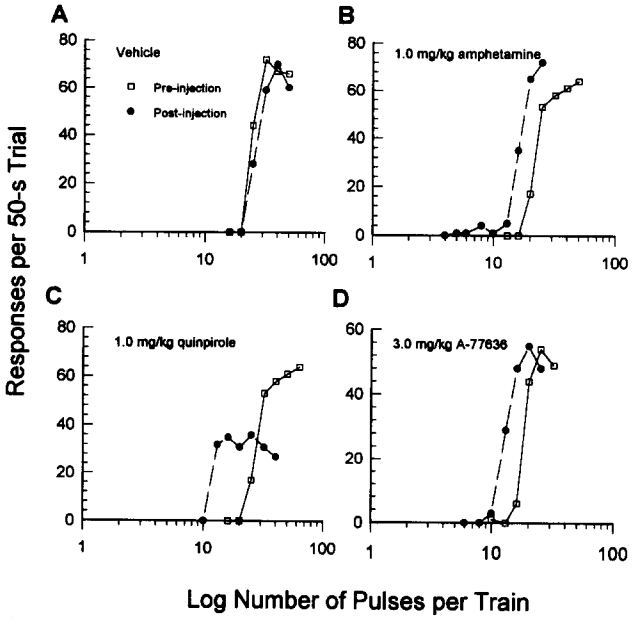


Fig. 2. Rate–frequency functions taken from one representative rat for each systemic treatment. Each graph depicts one rate–frequency function before (solid line and open square) and one after (broken line and filled circle) i.p. injection of (A) vehicle (distilled water, 1 ml/kg), (B) amphetamine (1.0 mg/kg), (C) quinpirole (1.0 mg/kg) or s.c. injection of (D) A-77636 (3.0 mg/kg). Rate–frequency functions were obtained by logarithmically decreasing the frequency of stimulation pulses from a value that sustained maximal lever pressing to one that failed to sustain lever pressing.

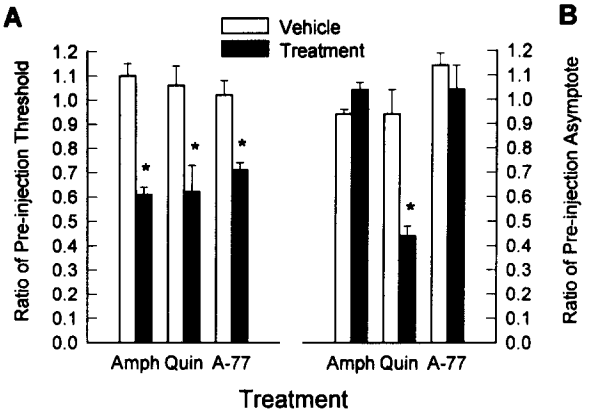


Fig. 3. Mean ratio of post- divided by pre-injection BSR thresholds (A) and asymptotes (B) for all rats receiving systemic amphetamine (1.0 mg/kg), quinpirole (1.0 mg/kg) or A-77636 (3.0 mg/kg). BSR thresholds and asymptotes were calculated using the Gompertz sigmoidal growth model [10] on the four rate–frequency functions obtained in the pre-injection session and all rate–frequency functions obtained during the first 30 min of testing (second 30 min of testing for the group receiving A-77636) in the post-injection session. Vertical bars represent S.E.M. *Signifies a threshold or asymptote value significantly different from the vehicle. Amph, amphetamine; Quin, quinpirole; A-77, A-77636.

compared to its vehicle. The largest change in asymptotic rates of responding occurred in the quinpirole group where the mean asymptote was reduced to 44% of the mean pre-injection value. Statistical analyses, consisting of a one-way ANOVA with repeated measures for threshold and a second ANOVA for asymptote data, were performed separately for each drug group.

The analyses on the ratios of pre-injection thresholds for each drug condition revealed a significant treatment effect in each group, $F_{1,5} = 49.29$, $F_{1,6} = 16.59$, and $F_{1,5} = 27.61$, all P's < 0.01 for amphetamine, quinpirole and A-77636, respectively. The significant treatment effects supported the observation that the three agonists reduced BSR thresholds and enhanced rewarding stimulation. Analyses of the asymptote data revealed a significant treatment effect only in the quinpirole group, $F_{1,6} = 27.61$, $P < 0.01$, indicating that, in this group, asymptotic rates of responding were significantly reduced by the drug.

Fig. 4 depicts representative rate–frequency functions obtained before and after microinjections of each dose of quinpirole into the caudal NAcc. It appeared that increasing doses of quinpirole produced progressively larger parallel shifts to the right of the rate–frequency function with little effect on asymptotic rates of responding. These rate–frequency functions were typical of those obtained from rats receiving microinjections of the four doses of quinpirole into the CP or cortex.

Fig. 5A depicts the mean ratios of pre-injection threshold after microinjections of increasing doses of quinpirole into the caudal NAcc (top), CP (middle) or

cortex (bottom). The top panel shows that ratios of pre-injection threshold were greater after intra-caudal NAcc injections of quinpirole than after intra-caudal NAcc injections of vehicle, an effect that was opposite to that seen in the group receiving systemic quinpirole injections (Fig. 3A). Moreover, the increase in threshold appeared to be larger in the groups receiving the higher doses of quinpirole than in those receiving smaller ones. Finally, these doses of quinpirole produced a pattern of increased threshold in the CP (middle) and cortex (bottom) that appeared indistinguishable from the one observed in the caudal NAcc (top). Hence, these data failed to demonstrate that the central quinpirole effect was specific to any of the three sites tested.

Fig. 5B illustrates the mean ratio of pre-injection asymptotes for all doses of quinpirole in each of the three injection sites. The data appear to show no consistent effects. Generally, for the caudal NAcc (top) the ratio of pre-injection asymptotes after quinpirole injections did not appear to differ from the corresponding value after vehicle injections. An exception may be noted in the group that received the 10.0 μg dose where the quinpirole ratio appeared to be lower than the vehicle ratio. The asymptote ratios in the groups receiving CP (middle) or cortex (bottom) injections showed similar patterns. That is, the quinpirole ratios of pre-injection asymptote did not appear to differ in a consistent manner from the vehicle ratios. Hence, quinpirole appeared not to change asymptotic rates of responding in any of the sites into which it was injected.

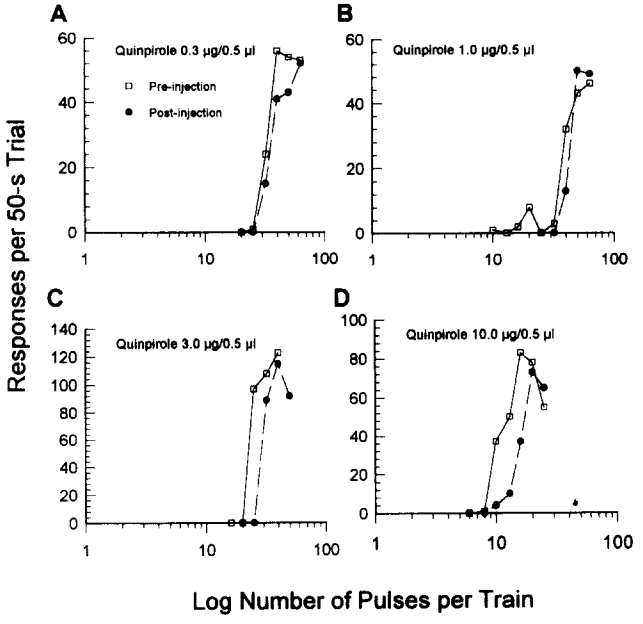


Fig. 4. Rate–frequency functions taken from one representative rat for each dose of quinpirole injected directly into the caudal NAcc. Each graph depicts one rate–frequency function before (solid line and open square) and one after (broken line and filled circle) intra-caudal NAcc microinjection of (A-D) 0.3, 1.0, 3.0 and 10.0 μg/0.5 μl of quinpirole. Rate–frequency functions were obtained by logarithmically decreasing the frequency of stimulation pulses from a value that sustained maximal lever pressing to one that failed to sustain lever pressing.

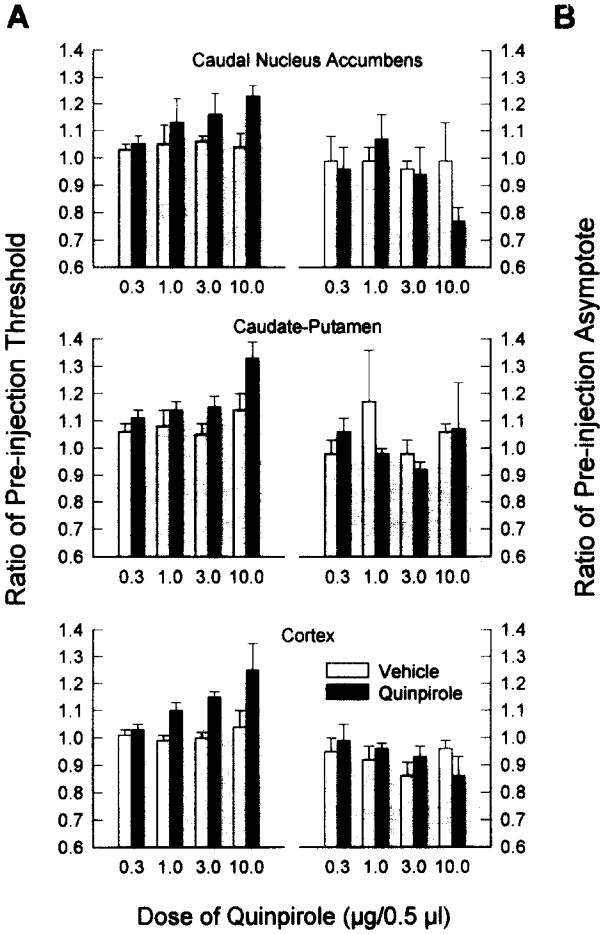


Fig. 5. Mean ratio of post- divided by pre-injection BSR thresholds (A) and asymptotes (B) for all rats receiving intracranial microinjections of quinpirole (0.3, 1.0, 3.0 or 10.0 μg/0.5 μl) in the caudal NAcc (top), CP (middle) or cortex (bottom). BSR thresholds and asymptotes were calculated using the Gompertz sigmoidal growth model on the four rate–frequency functions obtained in the pre-injection session and all rate–frequency functions obtained during the first 30 min of testing in the post-injection session. Vertical bars represent the S.E.M. Analysis of variance on the threshold data revealed a significant treatment effect for each site. Note the range of the vertical axis.

A 2-Way ANOVA was performed on the threshold data for each site separately. The CP groups receiving 0.3 and 3.0 μg contained the same subjects. This was the case also for the cortex groups. Hence, the 3.0 μg dose was excluded from the ANOVAs performed on data from these sites (this dose was chosen because its results lay on a point of the dose–effect function that provided the least new information). The analyses revealed a significant treatment effect for each site; $F_{1,14} = 14.51$, $P < 0.005$, $F_{1,12} = 14.51$, $P < 0.005$ and $F_{1,11} = 10.83$, $P < 0.01$ for the caudal NAcc, CP and cortex, respectively, and a significant dose effect for

the CP and cortex sites, $F_{2,12} = 5.05$, $P < 0.05$ and $F_{2,12} = 3.77$, $P < 0.05$, respectively. The analyses failed to reveal treatment × dose interactions. These results confirmed the observations of increased BSR thresholds with centrally administered quinpirole and failed to localize this effect. A 2-way ANOVA was performed on the asymptote data for each site separately (with the 3.0 μg dose excluded from the CP and cortex ANOVAs, see above). These analyses failed to show significant treatment or dose effects, supporting the observation that central quinpirole did not significantly change asymptotic rates of responding in any of the sites tested.

Fig. 6 depicts representative rate–frequency functions obtained before and after microinjections of A-77636 into the caudal NAcc or CP. The data illustrate that A-77636 in the caudal NAcc produced a parallel shift of the rate–frequency function to the left and decreased the asymptote (Fig. 6A). The data also show that A-77636 in the CP had no effect on the rate–frequency function (Fig. 6B).

Fig. 7A illustrates the mean ratios of pre-injection BSR thresholds in rats receiving A-77636 in the caudal NAcc or CP. The results show that intra-caudal NAcc injections of A-77636 resulted in a mean threshold that was lower than the pre-injection value, an effect that was not present after vehicle injections in the same site. Fig. 7A also shows that the mean ratio of pre-injection threshold after injection of A-77636 into the CP was not different from that obtained with vehicle injections into that site.

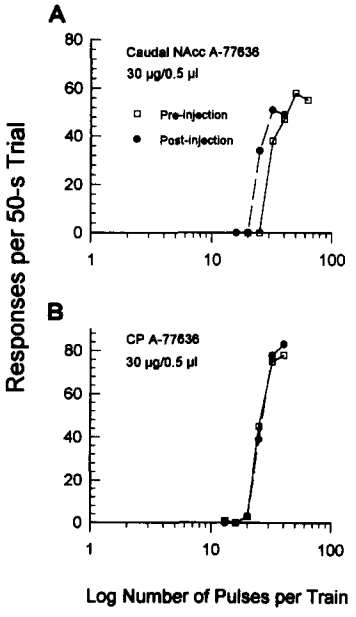


Fig. 6. Rate–frequency functions taken from one representative rat for each site into which intracranial microinjections of A-77636 were made. Each graph shows one rate–frequency function before (solid line and open square) and one after (broken line and filled circle) microinjection of 30.0 μg/0.5 μl of A-77636 into (A) the caudal NAcc or (B) CP. Rate–frequency functions were obtained by logarithmically decreasing the frequency of stimulation pulses from a value that sustained maximal lever pressing to one that failed to sustain lever pressing.

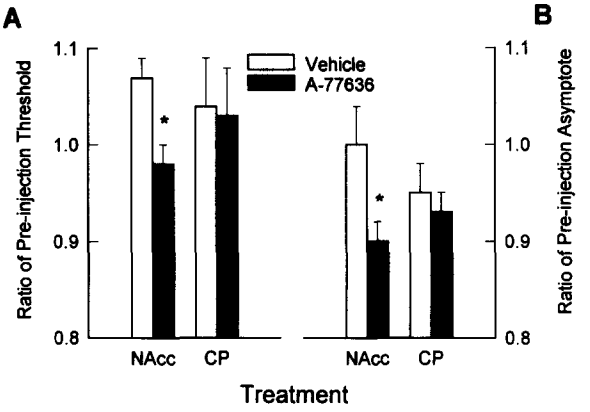


Fig. 7. Mean ratio of post- divided by pre-injection BSR thresholds (A) and asymptotes (B) for all rats receiving A-77636 (30.0 μg/0.5 μl) in the caudal NAcc or CP. BSR thresholds and asymptotes were calculated using the Gompertz sigmoidal growth model on the four rate–frequency functions obtained in the pre-injection session and all rate–frequency functions obtained during the first 30 min of testing in the post-injection session. Vertical bars represent the S.E.M. *Signifies a threshold or asymptote value significantly different from the vehicle. Note the range of the vertical axis.

Fig. 7B illustrates the mean ratio of pre-injection asymptote after intra-caudal NAcc and intra-CP injections of A-77636. The data show that asymptotic levels of responding were approximately 10% lower after intra-caudal NAcc injections of A-77636 than vehicle, an effect that was similar to systemic A-77636. In contrast, intra-CP injections of A-77636 had little effect on asymptotic responding.

A one-way ANOVA with repeated measures on the ratios of pre-injection threshold was performed on data from each group. A significant treatment effect was revealed in the group receiving intra-caudal NAcc injections of A-77636, $F_{1,6} = 27.61$, $P < 0.005$, indicating a reduction in the BSR threshold and enhancement of VTA rewarding stimulation. The analyses failed to reveal a significant treatment effect in the rats receiving intra-CP injections of A-77636.

A one-way ANOVA with repeated measures was performed on the asymptote data for each group receiving A-77636. The analyses revealed a significant treatment effect in the group receiving A-77636 in the caudal NAcc, $F_{1,6} = 6.01$, $P < 0.05$, supporting the observation of reduced asymptotic responding in this group. The analyses failed to reveal a significant treatment effect in the group receiving A-77636 in the CP.

4. Discussion

The present results showed that the systemic administration of amphetamine, quinpirole and A-77636 shifted rate–frequency functions to the left and re-

duced the threshold frequencies required to sustain responding for BSR. In each treatment the asymptotic rates of responding were not found to be greater in the post- than in the pre-injection period. Hence, it is unlikely that the reductions in BSR thresholds were caused by increased performance capacity of the rats. Rather, the reduced BSR thresholds after the systemic administration of each of the three DA agonists reflect a drug-produced potentiation of the rewarding efficacy of VTA stimulation.

Unlike the effects of systemic quinpirole, intra-caudal NAcc quinpirole resulted in shifts to the right of the rate–frequency functions and increased BSR thresholds. This effect was not accompanied by significant changes in asymptotic rates of responding. Injections of quinpirole into the CP and cortex were performed as an anatomical control for the effect observed in the caudal NAcc. The results showed similar increases in BSR thresholds with no significant reduction in asymptote. These results suggest that central quinpirole injections reduced the rewarding effects of VTA stimulation. The site in which this reduction occurred, however, failed to be specifically located to any of the regions tested.

Microinjections of A-77636 into the caudal NAcc were accompanied by shifts to the left of the rate–frequency functions and reduced BSR thresholds, effects that were similar to those observed with systemic injections of this agent. Intra-caudal NAcc injections of A-77636 were also accompanied by reductions in asymptote, suggesting that the reduced thresholds were not caused by some increased capacity of the rats to perform the required response. Rather, the reduced thresholds were most likely caused by an increase in the rewarding effects of VTA stimulation produced by A-77636. The failure to observe a significant change in threshold or asymptote when A-77636 was injected into the CP demonstrates the anatomical specificity of the caudal NAcc effect.

A reduction in BSR thresholds with systemic administration of amphetamine has been observed before [15,16,25]. Our results confirmed this effect and provide further support for the hypothesis that DA transmission is importantly involved in reward-related learning.

The present finding that systemic quinpirole reduced thresholds for VTA BSR is in accord with studies by Nakajima and colleagues [32,34] showing that systemic administration of quinpirole shifted rate–frequency functions to the left (enhanced reward) in rats responding for lateral hypothalamic BSR. These results corroborate studies that have demonstrated an enhancement of other types of reward with D_2-specific agonists [6,17,21,52]. Together these studies suggest that reward-related learning may involve stimulation of D_2 receptors.

The present finding that systemic injections of the novel D_1 specific agonist, A-77636, enhanced the rewarding effect of stimulation contrasts with the failure by Nakajima and O'Regan [34] to observe a similar effect with SKF 38393. These contradictory findings may result from the fact that SKF 38393 is a partial agonist [2,36]. In this regard, it would be interesting to evaluate the effects of central injections of SKF 38393 in the present paradigm.

The differential effects of systemic vs. central quinpirole suggest that the potentiative effects on BSR produced with systemic quinpirole occurred in a site(s) other than the NAcc, CP or cortex. In these sites central quinpirole injections *reduced* the rewarding effects of VTA self-stimulation. Further studies are needed to localize the brain stimulation reward-enhancing effects of quinpirole.

This present result, that central quinpirole reduced BSR, contradicts other studies that have investigated the effects on reward of intra-NAcc quinpirole. Thus, intra-NAcc injections of quinpirole potentiated responding for conditioned reward [55] and produced a conditioned place preference [52]. These differential findings may be a result of the different types of reward studied in the various paradigms and suggest that D_2 receptor stimulation in the NAcc does not participate in a facilitatory manner in all types of reward. For instance, there is evidence that DA transmission in the NAcc is required for conditioned reward [8,24,49,50], intravenous cocaine or opiate self-administration [12,46] and conditioned place preference [11,20] but not for food reward [1,13,46]. Similarly, it may be the case that NAcc D_2 receptor stimulation may be required for certain types of reward-related learning, but not for BSR.

The present finding, that A-77636 in the caudal NAcc enhanced BSR, was consistent with the systemic effects of this compound and is congruent with the rewarding effects of intra-accumbens SKF 38393 [52,55]. Together these studies suggest that D_1 receptor stimulation in the NAcc is importantly involved in brain stimulation- and other types of reward-related learning.

It has been previously suggested that a critical event in reward-related learning is a DA signal at the D_1 receptor [4,6,29,41]. This hypothesis leads to the prediction that a D_1 agonist would impair reward-related learning by masking the putative DA reward signal. In the present paradigm this would be manifested as an increase in the threshold of VTA rewarding stimulation. The present results are clearly in conflict with this hypothesis. It is possible that the unusually strong DA signal produced by VTA self-stimulation [38,39] failed to be masked with the present doses of A-77636, thus failing to increase thresholds. In this case, reward from

VTA stimulation may have been enhanced through a potentiation of the D_1 signal with A-77636.

Self- or forced-stimulation of the medial forebrain bundle, at the level of the lateral hypothalamus or VTA, has been associated with the release of DA in the NAcc as studied with in vivo microdialysis techniques [27,31,39]. The role of NAcc DA transmission in BSR is further demonstrated by reduced thresholds with intra-NAcc injections of amphetamine [9], an effect that appears to be localized to the caudal NAcc [42]. The present results suggest that perhaps VTA rewarding stimulation may occur through the action of DA at D_1 receptors in the caudal NAcc. Thus, increased D_1 receptor stimulation in the caudal NAcc with A-77636 produced an additive effect on VTA BSR.

The failure of intra-caudal NAcc quinpirole to enhance VTA BSR does not preclude a role of caudal NAcc D_2 receptors in this phenomenon. It may be the case that increasing D_2 receptor stimulation in the caudal NAcc above physiological levels does not add to VTA BSR but that normal D_2 receptor functioning in this site is necessary for reward-related learning to occur. We have collected data that support this hypothesis by showing increases in thresholds for VTA BSR after intra-caudal NAcc injections of a D_2 receptor antagonist (unpublished data). The increase in BSR thresholds with central injections of quinpirole remains difficult to explain.

The present results demonstrated that quinpirole potentiated VTA BSR when administered systemically but impaired it when injected directly into the caudal NAcc, CP or cortex. The results with systemic injections are in accord with other results showing that D_2 receptor stimulation is rewarding. However, they suggest that, in the case of VTA BSR, the rewarding effect may not occur through the action of DA at D_2 receptors in the caudal NAcc, CP or cortex. The present results showed that A-77636 enhanced VTA BSR when administered systemically or directly into the caudal NAcc. Although these findings do not completely rule out the possibility that rewarding stimulation of the VTA may involve a DA signal at the D_1 receptors, they argue more convincingly that VTA rewarding stimulation involves tonic activation of caudal NAcc D_1 receptors.

Acknowledgements

We would like to thank Steve Ferguson for his excellent technical assistance. We would also like to thank Smith, Kline and French Canada Ltd. for the generous gift of (+)-amphetamine, and Abbott Laboratories for their generous gift of A-77636. This research was funded by a grant from the Natural Sciences and Engineering Research Council of Canada to R.J.B.

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