Research Report

Rostral-caudal differences in effects of nucleus accumbens amphetamine on VTA ICSS

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Abstract

The effects of amphetamine along the rostrocaudal axis of the nucleus accumbens (NAcc) on ventral tegmental area (VTA) intracranial self-stimulation (ICSS) were studied. Eighteen rats were trained to lever press for ICSS in the VTA. Rate-frequency functions were determined by logarithmically decreasing the frequency of cathodal pulses in a stimulation train from a value that induced maximal responding to one that induced no responding (thresholds). After ICSS thresholds stabilized, (+)-amphetamine (20.0 μg/0.5 μl) or its vehicle, distilled H₂O (0.5 μl), were injected directly into the rostral NAcc (n = 6) or the caudal NAcc (n = 8) or the caudate-putamen (CP) (n = 5) just dorsal to the caudal NAcc. Results showed that amphetamine in the caudal NAcc significantly decreased ICSS thresholds without affecting asymptotic rates of responding, indicating a potentiation of the rewarding efficacy of VTA stimulation. Amphetamine in the rostral NAcc or CP produced smaller, non-significant, decreases in ICSS thresholds. Further analyses revealed a significant positive correlation (r = 0.51, P < 0.05) between the site of injection along the rostrocaudal axis of the NAcc and the size of the amphetamine-produced potentiation of VTA stimulation reward. Others have reported topographical differences, including dopamine terminal density and D₁ receptor density, in the NAcc. The present results indicate that these anatomical and neurochemical differences appear to be correlated with behavioural differences.

Key words: Amphetamine; Dopamine; Intracranial self-stimulation; Reward; Reinforcement; Nucleus accumbens; Caudate-putamen; Curve shift

1. Introduction

Studies aimed at delineating the neural substrates of reward-related learning have revealed a strong role for dopamine (DA) transmission (for detailed reviews see [2] and [67]). Thus, DA agonists decreased intracranial self-stimulation thresholds (ICSS) [20,25,47], increased responding for conditioned reward [7,13,40,54], produced conditioned place preferences [21,27,29,30,66] and were intravenously self-administered [51,59,70]. Accordingly, DA antagonists produced extinction-like declines in responding for ICSS [19] and food reward [4,68], reduced the rewarding effects of intravenously self-administered cocaine [15,35,37,55] and amphetamine [72], blocked place preferences based on DA agonists [42,65] and attenuated responding for conditioned reward [6,28,53]. These studies suggest that DA transmission is importantly involved in the ability of rewarding stimuli to control behaviour.

In vivo neurochemical studies have shown that DA is released in the nucleus accumbens (NAcc) [10,26,33,45,52] and caudate-putamen (CP) [10,33,34] of rats that are presented with primary or conditioned rewarding stimuli. These findings suggest that DA transmission in these brain regions may constitute an element of the reward process. This hypothesis is supported by reports that intra-NAcc and intra-CP injections of DA agonists and antagonists facilitate and inhibit, respectively, reward-related learning [5,8,11,18,38,41,60,66]. It remains to be determined how the release of DA in these structures participates in the putative neuronal modifications that occur in reward-related learning.
Recent anatomical and biochemical studies have begun to characterize in detail the heterogeneous nature of the NAcc. It seems that this region of the forebrain can be divided into two compartments, referred to as 'core' and 'shell' based on the differential arrangement of intrinsic cells and afferent and efferent projections [24,73]. The NAcc also has been shown to possess a rostrocaudal gradient of increasing DA terminal density as well as the D_{1}-subtype of dopamine receptors [1]. Finally, ventral tegmental area (VTA) DA neurons projecting to the caudal, but not the rostral NAcc, release cholecystokinin (CCK) as a co-neurotransmitter [31]. The rostrocaudal anatomical and neurochemical differences may lead to a rostrocaudal difference in the role of the NAcc in reward-related learning. Thus, the rate at which animals respond for reward may be more sensitive to dopaminergic manipulations of the caudal than of the rostral NAcc. The present experiment tested this hypothesis.

Rats were trained to self-stimulate the VTA, a DA-rich region of the midbrain [9], and microinjections of amphetamine were made into the rostral or caudal NAcc as well as the CP. With the use of a psychophysical method referred to as the curve-shift paradigm [43], the effects of amphetamine on self-stimulation thresholds were evaluated.

2. Materials and Methods

Treatment of 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 18 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 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 in the home cages ad libitum.

2.2. Apparatus

The experimental environments consisted of four similarly constructed operant chambers measuring 29 × 23 × 18 cm high. The chambers were constructed of aluminum sides and plexiglass backs, tops and doors. The top of each chamber contained a hole 3 cm in diameter to allow the stimulation lead to pass into the chamber. 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. The onset and offset of the lights signified the start and end of a trial, respectively.

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

2.3. Surgical procedure

The rats were anaesthetized with sodium pentobarbital at 65 mg/kg of body weight and fitted into the stereotaxic apparatus. Moveable electrodes [42] were implanted in the VTA using the stereotaxic coordinates of −4.8 mm from 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 using the corresponding coordinates of 0.8, 1.5 and 7.2 or the rostral NAcc at 2.2, 1.5 and 7.0–7.2 mm. The coordinates for the CP cannula guides were 0.8, 1.8 and 5.2 mm. The incisor bar was positioned at 3.5 mm below the horizontal plane passing through the interaural line [48].

Electrodes consisted of a plastic guide and a moveable stainless steel wire (0.25 mm in diameter) coiled with ephylite, except for the tip. The cannula guides (0.64 mm diam.) were made from modified 23-gauge stainless-steel needles. The injectors were made from stainless-steel tubing (0.32 mm diam) to achieve a length of 1 mm longer than the cannula guides. A stainless-steel wire (0.32 mm diam.) was kept in the guide cannula between injections.

2.4. Procedure

Following more than one week of postoperative recovery the rats were tested for ICSS using 0.3 s trains of cathodal rectangular pulses each lasting 0.1 ms in duration. During the response-shaping period the current intensity and frequency were varied manually and finally fixed at parameters that maintained bar pressing. The rats then were allowed to press the lever freely for 1 h daily for 4 consecutive days. ICSS thresholds then were determined by setting the frequency of pulses at the value that induced maximal responding and decreasing the frequency by 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 (F10.5) for trials of 50 s with an intertrial interval of 15 s. Presses on the rewarded lever were counted for each trial.

The testing period began when the ICSS threshold was 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 [16]) did not differ from the mean threshold by more than 10 percent.

The testing period consisted of individual test sessions each separated by at least 48 h. Each rat was tested with one or more dopaminergic agents microinjected into any particular site and/or 1 to 4 systemic injections, the order of treatment being different for each rat. However, only the central vehicle (distilled H_{2}O) and (+)-amphetamine (20 μg in 0.5 μl) data are reported here. Test sessions began with 2 new determinations of ICSS threshold after which the rat was removed from the operant chamber. Rats were fitted with an injection cannula connected to a 10-μl Hamilton microsyringe 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. In all conditions the rats were returned to the operant chamber and new determinations of ICSS thresholds were obtained for 30 min.

2.5. Histology

When the testing period was over the rats were exsanguinated with saline, perfused with formalin and decapitated. The brains were removed and placed in 10% formalin for a minimum of 7 days. The brains then were cut in 40 μm serial sections and stained with thionin in order to verify the actual implantation sites.

2.6. Data analyses

Data gathered from the pre- and post-treatment portions of each session were curve-fitted and threshold and asymptote estimates
were obtained using the Gompertz sigmoidal model. The post-treatment threshold value was divided by the pre-treatment threshold value to obtain a ratio. This calculation was performed for the vehicle and amphetamine conditions. A 2-way analysis of variance (ANOVA) with independent groups (rostral NAcc, caudal NAcc and CP) and repeated measures on the treatment factor (vehicle vs. amphetamine) then was carried out. A significant treatment effect would indicate a potentiation of VTA ICSS reward produced by amphetamine. A significant treatment by group effect would indicate that the amphetamine-produced potentiation of ICSS depended on the site in which it was injected.

A correlation between the site of injection in the NAcc and the size of the amphetamine effect also was calculated. For this analysis the value representing the size of the amphetamine effect was obtained by subtracting the post- to pre-treatment threshold ratio of the vehicle condition from the same ratio of the amphetamine condition.

3. Results

Histological analyses revealed that the tips of all the stimulating electrodes were located in or just anterior to the VTA (Fig. 1C). Fourteen of the injector tips were distributed across the rostral-caudal axis of the NAcc (Fig. 1A). The remaining 5 injector tips were located in the CP dorsal to the caudal aspect of the NAcc (Fig. 1B). One rat received both rostral NAcc and CP injections.

Fig. 2 shows rate–frequency functions obtained from three rats each with a cannula aimed at one of three sites; the caudal NAcc, the rostral NAcc or CP. Fig. 2A shows rate–frequency functions for the vehicle condition. In all three sites the post-vehicle rate–frequency functions appeared to be shifted slightly to the right of the pre-vehicle functions. The degree of rightward shift did not seem to differ with site of injection. Fig. 2B shows rate–frequency functions for the amphetamine condition. In the caudal NAcc amphetamine appeared to shift the lateral position of the rate–frequency function to the left, previously ineffective frequencies now producing responding. In the rostral NAcc and CP amphetamine also tended to shift the rate–frequency function to the left but to a much lesser degree than in the caudal NAcc. Amphetamine injections did not appear to change asymptotic levels of responding.

Fig. 3 depicts the mean (±S.E.M.) ratio of baseline ICSS thresholds for the vehicle and amphetamine conditions in each of the three groups. In the caudal NAcc ICSS thresholds obtained after injections of amphetamine were lower than those obtained after injections of vehicle. In the rostral NAcc and CP amphetamine produced ICSS thresholds that were only slightly lower than those after vehicle.

These observations were supported by the statistical analyses. The 2-way treatment by injection site ANOVA revealed a significant interaction, \( F_{2,16} = 10.39, P < 0.005 \). Tests of simple main effects revealed a significant treatment effect in the caudal NAcc, \( F_{1,16} = 43.75, P < 0.001 \). These analyses indicated that amphetamine in the caudal NAcc, but not the rostral NAcc or the CP, decreased ICSS thresholds to levels significantly
Fig. 2. Rate-frequency functions taken from one representative rat for each site of injection. Each graph shows one rate-frequency function before (solid lines) and one after (broken lines) microinjection of (A) 0.5 μl of vehicle or (B) 20.0 μg/0.5 μl of amphetamine into the caudal NAcc (top), rostral NAcc (middle) or CP (bottom). Rate-frequency functions were obtained by decreasing the frequency of stimulation pulses from a value that sustained maximal lever pressing to one that failed to sustain lever pressing.

less than those obtained with the vehicle. A similar 2-way ANOVA on asymptotic responding (data not shown) revealed no significant effects, indicating that amphetamine did not significantly change asymptotic levels of responding in any of the groups.

Fig. 4 illustrates the size of the amphetamine effect as a function of rostrocaudal site of injection. The size of the amphetamine effect was calculated by subtracting the ratio of baseline ICSS threshold for the vehicle condition from the equivalent ratio for the amphetamine condition for each animal receiving injections in the rostral or caudal NAcc. The data show that amphetamine's reward-enhancing effect increased linearly with increasingly more caudal injections. To further explore this linear relationship a correlation coefficient relating the two variables was calculated. The analysis revealed a significant correlation between rostral-caudal site of amphetamine injection and the size of the amphetamine effect, $r_{13} = 0.51, P < 0.05$.

4. Discussion

Microinjections of vehicle into the NAcc and CP were accompanied by small shifts to the right of the
rate–frequency functions as well as a small increase in ICSS thresholds. These data are indicative of a small attenuation of the rewarding efficacy of VTA ICSS. These results probably did not result from the vehicle injections per se since a similar increase in ICSS thresholds was observed in 5 rats that received no injection (data not shown). Rather, the data suggest that the reward substrate becomes less sensitive as the animals continue to self-stimulate. Similar observations have been made by other investigators [56,57] using a similar protocol.

Microinjections of amphetamine into the caudal NAcc significantly decreased thresholds for VTA ICSS without affecting asymptotic levels of responding. This finding is consistent with that of Colle and Wise [14] using lateral hypothalamic ICSS. In their study the location of the injector tips within the NAcc was not reported. Our finding that microinjections of amphetamine into the CP just dorsal to the caudal NAcc failed to enhance VTA ICSS also is in accordance with the Colle and Wise study [14] and demonstrates the anatomical specificity of the caudal NAcc effect. The present results corroborate an extensive literature showing that the NAcc plays an important role in reward-related learning [36,46,67] and suggest that, at least in regards to VTA ICSS, the caudal portion of the NAcc plays a more important role than the rostral portion.

Amphetamine increases the neurogenic release of DA and blocks its reuptake [58,63]. This suggests that amphetamine enhanced the rewarding effects of VTA stimulation through dopaminergic mechanisms in the caudal NAcc. Although the present study does not provide evidence to rule out the possibility that amphetamine acted on noradrenergic mechanisms, this question has been addressed by Colle and Wise [14] who injected L-amphetamine, a compound that has the same chemical properties as d-amphetamine except for a greatly reduced DA-releasing action [23,32], into the NAcc and found no effects on ICSS. Hence, it is most likely that the release of DA, and not norepinephrine, in the caudal NAcc plays an important role in VTA ICSS.

Others have reported regional differences in the NAcc. These include a greater density of DA terminals and D₁ receptors [1] in the caudal than in the rostral NAcc. It also has been shown that in the caudal NAcc CCK and DA are co-localized whereas in the rostral NAcc they are not [31]. The present findings indicate that these anatomical and neurochemical differences are correlated with behavioural differences, viz., sensitivity to amphetamine-produced potentiation of VTA ICSS. These results suggest several possible underlying mechanisms as determining factors in the role of the NAcc in reward. Some of these may include the degree of DA release, the degree of D₁ receptor stimulation and the co-release of CCK. The present experiment tested whether the degree of DA release played a role in VTA ICSS and found that it does. Data relevant to the other hypotheses have been reported by others.

The hypothesis that the release of DA in the NAcc is involved in reward is supported by in vivo chronomperometric and microdialysis studies showing an increase over baseline levels of DA in the NAcc when rewarding pulses are self- or experimenter-administered to the VTA [22,50]. Compelling evidence for a role of NAcc DA release in reward is a positive correlation demonstrated between rate–intensity functions and DA levels in the NAcc during VTA ICSS [49]. In light of these findings the present study suggests that amphetamine may have produced its greatest enhancement of VTA ICSS in the caudal NAcc because of the greater amount of DA available in this region.

The greater sensitivity of the caudal NAcc to amphetamine-produced enhancement of VTA stimulation may be related to the greater density of D₁ receptors in this region [1]. Perhaps the degree of D₁ receptor stimulation is a determining factor in reward. It has been hypothesized by several authors that D₁ receptors play a crucial role in reward-related learning [3,44,46,64,71] and a role for NAcc D₁ receptors in VTA ICSS reward [38] as well as other types of reward [12,66,69] has been observed. Confirmation of this hypothesis awaits further study.

Studies investigating the effects of CCK on self-stimulation also have revealed behavioural differences between the rostral and caudal NAcc in the rat. Microinjections of CCK into the rostral NAcc antagonized self-stimulation [17,61] while injections into the caudal NAcc potentiated it. This is in excellent agreement with another study showing that injections of the CCK antagonist, proglumide, into the caudal NAcc significantly antagonized VTA ICSS whilst, when injected into the rostral NAcc, it produced weak facilitatory effects [62]. It has been shown that CCK interacts with DA differentially across the rostrocaudal axis of the NAcc [39]. Perhaps these differential effects on DA systems are related to the behavioural differences produced with CCK and proglumide. This differential CCK-DA interaction across the rostrocaudal axis of the nucleus accumbens may be related to the differential amphetamine effect observed here.

The present study investigated the role of DA in the NAcc in VTA ICSS. The results indicated that the rewarding effects of VTA stimulation were potentiated when amphetamine was injected directly into the caudal NAcc but not when it was injected into the rostral NAcc or the CP. These results are in accord with the finding that DA is released in the NAcc when animals are responding for VTA rewarding stimulation as well as other types of reward. Furthermore, the results suggest that DA release in the caudal NAcc is an
important factor in the role of the NAcc in reward and show that the rostrocaudal anatomical and neurochemical differences that exist in the NAcc appear to be correlated with behavioural differences.

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6. References

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