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Dopamine Mediates Ipsi- and Contraversive Circling Elicited from the Substantia Nigra

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VACCARINO, F. J. AND K. B. J. FRANKLIN. *Dopamine mediates ipsi- and contraversive circling elicited from the substantia nigra.* PHARMAC. BIOCHEM. BEHAV. 17(3) 431–434, 1982.—It was found that ipsiversive and contraversive circling could be induced by imposed stimulation of the lateral and medial portions of the substantia nigra pars compacta (SNC), respectively. Stimulation of more ventral sites in the substantia nigra (SN) did not elicit circling. Pimozide, a dopamine (DA) antagonist, dose dependently blocked both ipsiversive and contraversive circling induced by imposed stimulation of the SN, but did not alter circling elicited from the non-dopaminergic cerebral peduncle. However, amphetamine, a catecholamine agonist, did not facilitate stimulation-induced circling elicited from the SN possibly because it releases DA on both sides of the brain and thus fails to exaggerate the imbalance of activity produced by stimulation. Taken together these results suggest that DA is involved in SN mechanisms mediating both ipsiversive and contraversive stimulation induced circling and provide further evidence that the lateral SN may be functionally antagonistic to the medial SN.

Substantia nigra Ipsiversive circling Contraversive circling Dopamine Pimozide Amphetamine

STUDIES investigating nigrostriatal-mediated circling have demonstrated that rats with unilateral substantia nigra (SN) lesions will circle contraversively following apomorphine and ipsiversively following amphetamine [2,8]. Amphetamine-induced ipsiversive circling is believed to be due to preferential release of dopamine (DA) from nigrostriatal fibres on the intact side while apomorphine is thought to preferentially activate supersensitive DA receptors on the lesioned side [2,8]. This has been interpreted to suggest that rats will circle away from the more active striatum. However, recently it has been reported that when lesions were restricted to the lateral portions of the SN, apomorphine produced ipsiversive circling [1,7]. Re-examination of data presented by Hodge and Butcher [4] reveals a similar trend though they did not comment on it. These results suggest that the SN may not be functionally homogeneous with respect to circling mechanisms.

In support of this notion, a recent study found that amphetamine produced ipsiversive circling in rats with medial SN lesions but contraversive circling in rats with lateral SN lesions [9]. In addition, it was found that electrical stimulation of the medial SN produced contraversive circling while stimulation of the lateral SN produced ipsiversive circling [9]. These findings were taken to suggest that lateral SN mechanisms are antagonistic to medial SN mechanisms driving contraversive circling.

It is possible, however, that circling elicited by electrical stimulation of the SN is not dependent on DA neurons but on other groups of neurons such as GABA neurons which pass through and synapse in this region [5]. To determine the extent to which circling elicited by imposed electrical stimulation of the SN is DA dependent, this experiment investigated the effects of d- and *l*-amphetamine and pimozide on circling elicited from either medial or lateral SN sites. Since d-amphetamine is more potent at stimulating behaviors which depend on DA [3, 6, 10] than *l*-amphetamine and pimozide is a DA receptor blocker, it would be expected that d-amphetamine would facilitate circling more than *l*-amphetamine and pimozide would block circling if DA was involved. In addition, electrode tips were aimed at various SN sites in order to find out if the circling was elicited only from sites near the DA cells of the pars compacta.

METHOD

Subjects

Subjects were 30 male hooded rats obtained from Charles River Canada Inc. They were housed in 14×25×47 cm plastic cages on a twelve hour diurnal cycle. Food (Purina Rat Lab Chow) and water were available ad libitum throughout the experiment. The rats weighed 250–350 g at the time of electrode implantation.

Apparatus

Circling was observed in a galvanized metal cylinder (radius 14.5 cm; rotation cylinder). During the testing the stimulation apparatus was attached to the rat's implanted electrode via a lead and a mercury commutator (Mercotac). The apparatus was designed to deliver 0.2 sec trains at the rate of 4/sec. Each 0.2 sec train consisted of 0.2 msec, 100 Hz monophasic rectangular pulses. Pilot studies showed that

these stimulation parameters maximized circling without affecting direction. The stimulation trains were controlled by solid state programming apparatus (Coulbourne, Lehigh Valley), and monitored on an oscilloscope (Tetronix, Type 502) connected across a 100 ohm series resistor. A 0.1 μF capacitor was connected in series with the rat to prevent electrode polarization.

Drugs

D- and *l*-amphetamine sulphate were a gift from Smith, Kline and French, Canada Ltd. and were administered in a volume of 1 ml/kg with 48 hrs separating each drug test. D- and *l*-amphetamine sulphate were dissolved in a 0.9% saline solution and administered IP. Pimozide was administered SC in a vehicle of 3% tartaric acid formula in a volume of 1 ml/kg. Seventy-two hrs separated pimozide drug tests. All doses are calculated as the salt.

Surgery

Rats were anesthetized for surgery with approximately 50 mg/kg of sodium pentobarbital (Nembutal). A twisted bipolar electrode (Plastic Products Ltd.) 127 μm in diameter was implanted using a Stoelting Co. stereotaxic instrument. Electrode tips were aimed at the substantia nigra pars compacta (SNC) of the right hemisphere of each animal. Rats were randomly divided into "medial" and "lateral" groups. These groups were subdivided on the basis of the histological findings (see Results). Co-ordinates for the medial group were: 3.2 mm posterior to bregma, 1.9 mm lateral to the midline suture and 8.6 mm ventral to the dorsal surface of the skull. Co-ordinates for the lateral group were 3.2 mm posterior to bregma, 2.6 mm lateral to the midline suture and 8.0 mm ventral to the dorsal surface of the skull. The nose bar was set 5 mm above the interaural line.

Procedure

The current levels used to induce circling were 100, 200 and 300 μA. Pilot tests revealed that the upper and lower ranges of circling rate were elicited within these boundaries at the stimulation parameters used.

Ten days following surgery (Day 1) the drug tests began. All rats received both amphetamine and pimozide treatment in counterbalanced order. In the amphetamine condition, on Days 1, 3, 5 and 7 all rats were tested under four drug conditions: saline vehicle, 1 mg/kg d-amphetamine, 1 mg/kg *l*-amphetamine or 2 mg/kg *l*-amphetamine. Each rat received the drugs in a different random order. Rats were placed in the rotation cylinder immediately following drug administration. Twenty-five min later, rats were observed for rate and direction of circling for 1 min with no stimulation. Stimulation at one of the three current levels was then applied for 1 min; rate and direction of circling were noted. Five and 10 min later rats were tested at the remaining two current levels. Order of current levels was randomized for each animal.

In the pimozide condition, on Days 1, 4 and 7 all rats were tested under three drug conditions: 0.3 mg/kg pimozide, 1 mg/kg pimozide or tartaric acid vehicle. Each rats received drugs in a different random order. Three and one-half hours following drug injection, rats were placed in the rotation cylinder and observed for stimulation induced circling starting 4 hrs post-injection using the same procedure described in the amphetamine condition.

All rats were then allowed 7 days free from testing. Beginning on Day 14 the entire procedure was repeated, the amphetamine treated rats now receiving pimozide and vice versa.

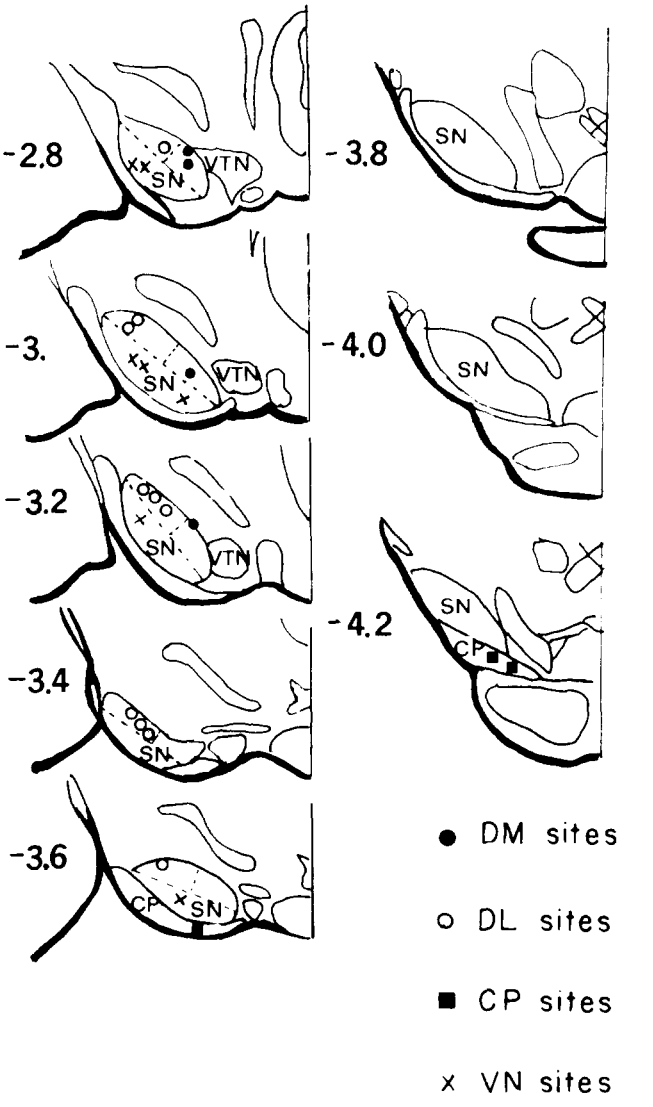

FIG. 1. Electrode site distribution of DM, DL, VN and CP groups. The dorsolateral and ventral SN borders are depicted by the broken lines. Anterior-posterior levels from bregma are given. Legend: VTN—ventral tegmental nucleus; CP—cerebral peduncle; SN—substantia nigra.

Histological Procedure

Following testing, animals were anesthetized with sodium pentobarbital (100 mg/kg) and perfused first with 0.9% saline and then with 10% Formalin. The brains were removed and kept in 10% Formalin for two days. Forty μm frozen sections were cut and were stained with thionin for microscopial examination.

RESULTS

Based on the histological examinations, rats were divided into 4 groups: a dorsomedial group (DM), with electrode tips located in the medial half of the SNC (n=4); a dorsolateral

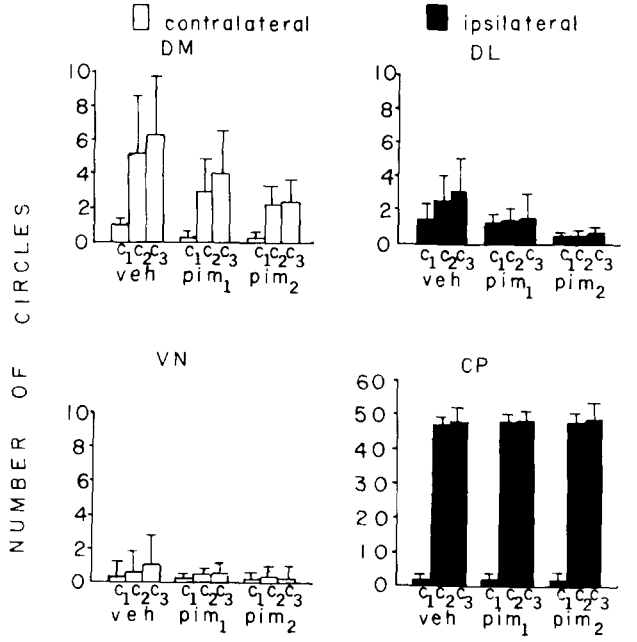

FIG. 2. The magnitude and direction of stimulation-induced circling in the dorsomedial SN group (DM), the dorsolateral SN group (DL), the ventral SN group (VN) and the cerebral peduncle group (CP) following treatment with the vehicle (veh) and two pimozide doses; 0.3 mg/kg (pim_1) and 1.0 mg/kg (pim_2). Circling was measured during one minute tests at each of the following three current levels: 100 μA (C_1), 200 μA (C_2) and 300 μA (C_3). Vertical bars represent standard errors of the means.

group (DL), with electrode tips located in the lateral half of the SNC (n=11); a ventral group (VN), with electrode tips located in the ventral half of the SN, the pars reticulata (SNR) (n=7); and a cerebral peduncle group (CP), with electrode tips located ventral of the SNR in the cerebral peduncle (n=3). Figure 1 shows the anatomical divisions used and the distribution of the electrode tips in the 4 groups. The remaining 5 rats had electrode tips outside these areas and were not included in the statistical analyses.

All statistical analyses were based on a square root transformation of the data; this was done to reduce the heterogeneity of variance caused by the mean circling rates being proportional to the variances.

Stimulation Induced Circling Following Amphetamine

A 4×4×3 analysis of variance comparing group × drug × current level revealed no group × drug effect. None of the amphetamine treatments had any significant effect on circling magnitude or direction, $F(9,63)=0.21$, N.S. There was, however, a significant group effect showing that the DM group circled contraversively, the DL and CP groups circled ipsiversively, and the VN group showed no direction preference, $F(3,22)=18.28$, $p<0.0001$. In addition, there was a significant group × current level effect showing that increasing the current level increased the magnitude of ipsiversive circling in the CP and DL groups and increased contraversive circling in the DM group but had no effect on the VN group, $F(6,42)=39.70$, $p<0.0001$.

Stimulation Induced Circling Following Pimozide Treatment

A 4×3×3 way analysis of variance comparing group × drug × current level revealed a significant group × drug effect and a significant group × current effect. When current was increased the magnitude of contraversive circling in the DM group increased, the magnitude of ipsiversive circling in the DL and CP groups increased, while no circling was seen in the VN group, $F(6,42)=43.49$, $p<0.0001$.

The significant group × drug effect showed that circling was reduced in the DM and DL groups in a dose related manner following 0.3 mg/kg and 1 mg/kg of pimozide, $F(6,42)=3.63$, $p=0.0054$. None of the pimozide doses affected circling in the CP group.

A three-way group × current × drug effect was also found. This was attributed to the fact that although circling observed in DM, DL and CP groups was increased with increasing current, pimozide reduced circling in only the DM and DL groups, and not the CP group, $F(12,84)=2.45$, $p=0.0086$.

Figure 2 shows a summary of these pimozide results.

DISCUSSION

Results from this experiment are consistent with the findings from our previous experiment investigating medial-lateral differences in stimulation-induced circling [9]. DM rats consistently circled contraversively and DL rats consistently circled ipsiversively with stimulation. The fact that DM and DL rats showed stimulation-induced circling, while VN rats showed no significant circling indicates that SN stimulation must occur in the vicinity of DA cell bodies in order to induce circling with the stimulation parameters used in this experiment.

Further support for the involvement of DA in SN circling mechanisms comes from the finding that pimozide dose-dependently antagonized both ipsiversive and contraversive circling. These effects are not likely due to non-specific motor deficits of akinesia since pimozide had no effect on the circling observed following stimulation of the cerebral peduncle, a non-DA fibre system.

It was, however, surprising to find that neither d- nor *l*-amphetamine at any of the doses used, produced any significant increase of stimulation induced circling in the SN. Amphetamine was expected to be ineffective in the CP group, since the cerebral peduncle is a non-catecholamine fibre system. It is difficult to reconcile the above amphetamine findings with other findings in this experiment which suggest that DA is important in mediating stimulation-induced circling in the SN. However, since amphetamine did not increase circling magnitude above that which was achieved by stimulation alone, it can be inferred that neither d- nor *l*-amphetamine increased the nigrostriatal DA imbalance above that which was achieved by stimulation alone. A possible explanation for this may be that, because amphetamine was administered peripherally, central striatal DA release was increased by the same proportions bilaterally. In other words, amphetamine may be increasing nigrostriatal DA activity bilaterally without increasing the nigrostriatal imbalance caused by stimulation.

The present findings support previous findings suggesting that there are two functionally distinct and possibly antagonistic SN systems mediating circling behavior. The present data, however, do not make clear at what level of nigrostriatal functioning the medial and lateral mechanisms involved in circling are manifest. In the only study to date

addressing this issue, Thal *et al.* [7] investigated striatal binding characteristics of their ipsiversive and contraversive circlers. They found increases in striatal [^{3}H] spiroperidol and [^{3}H] haloperidol following both medial binding and lateral lesions. However, no differences in binding parameters were found between the two groups, suggesting that the differential effects of apomorphine following medial and lateral SN lesions could not be attributed to DA receptor differences between the two groups. It is unclear, then, where and how the site-dependent differences found in this study become operative. This may depend on which cells are stimulated, but direct evidence remains to be obtained.

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