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Neuropeptide Y: Intrastriatal Injections Produce Contralateral Circling That is Blocked by a Dopamine Antagonist in Rats[1]

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MOORE, E., Z. MERALI AND R. J. BENINGER. *Neuropeptide Y: Intrastriatal injections produce contralateral circling that is blocked by a dopamine antagonist in rats.* PHARMACOL BIOCHEM BEHAV **48**(3) 681–688, 1994.—The brain is rich in neuropeptide Y (NPY) but its function is poorly understood. Previous studies have shown that intrastriatal injections of NPY stimulate dopamine (DA) release. In the present paper, behavioral studies evaluated the possibility that unilateral intrastriatal injections of NPY would produce contralateral circling that could be blocked by coinjection with a DA antagonist. Four experiments examined circling behavior in rats after unilateral intrastriatal microinjections (0.5 μl) of: 1) amphetamine alone; 2) amphetamine with the DA antagonist *cis*-flupenthixol; 3) NPY alone; and 4) NPY with *cis*-flupenthixol. Each experiment consisted of seven test sessions; the first and seventh were preceded by no injection, the second and sixth by a control injection (saline or *cis*-flupenthixol with saline) and the third, fourth, and fifth by drug injections. Animals were scored during two 5-min intervals of a 20-min test session that began with the central injection and placement in a circular arena (30 cm diam.). Results indicated that the 25.0- but not the 6.0- or 12.0-μg doses of amphetamine and the 0.10- but not the 0.01- or 1.0-μg doses of NPY produced contralateral circling. This directional bias was antagonized by *cis*-flupenthixol (20 μg in 0.5 μl) in the case of amphetamine and fully blocked in the case of NPY. Results raise the intriguing possibility that contralateral circling induced by unilateral intrastriatal NPY may be mediated by DA.

Circling Neuropeptide Y Striatum Dopamine *cis*-Flupenthixol Amphetamine

NEUROPEPTIDE Y (NPY), a member of the pancreatic polypeptide family, consists of 36 amino acids. It was first isolated and chemically characterized in the porcine brain (33, 34). NPY is widely distributed in the human (1,9) and rat brain (2,11,13,26) and NPY receptors exist in brain membranes of the rat (29,35) and human (37). Of particular interest to the present studies is the finding of abundant NPY cell bodies and fibres in the striatum (31).

The behavioral functions of NPY are still poorly understood, but NPY now has been implicated in a number of behavioral phenomena. It was found that intrahypothalamic NPY induced feeding (36), NPY injected locally into several limbic structures influenced memory (12), and a recent report implicated nucleus accumbens NPY in reward-related learning (19). Of particular interest for the present studies is the finding that ICV NPY produced decreases in locomotor activity in rats (14,16,17).

Neurochemical studies of postmortem brain tissue from rats treated with ICV NPY have revealed increased levels of striatal dopamine (DA) and its metabolites, suggesting increased synthesis and release (15). Others have found that NPY injected directly into the striatum produced similar effects on DA release (3). These findings may make the observation that ICV NPY decreased locomotor activity surprising because manipulations that increase striatal DA reliably produce increases in locomotor activity (4). One possibility is that ICV NPY influences brain regions that are downstream from striatal output where they might reverse the usual motor effects of increases in striatal DA.

The present experiments examined the hypothesis that in-

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trastriatal NPY would produce enhanced locomotor activity and that this effect would be blocked by a DA receptor antagonist. It has been shown reliably that following unilateral stimulation of striatal DA, rats circle away from (contralateral to) the side of higher DA activity (27,28). Circling behavior was evaluated in a series of four experiments. Experiment 1 was carried out to demonstrate that a known DA enhancer, amphetamine, would produce contralateral circling in the present paradigm when injected unilaterally into the dorsal striatum and Experiment 2 determined whether the DA antagonist, *cis*-flupenthixol, would block this effect. Experiment 3 examined the effects of intrastriatal NPY, and contralateral circling was observed. Experiment 4 determined whether *cis*-flupenthixol would block this effect.

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 Animal Care Committee of Queen's University.

Animals

Male albino Wistar rats weighing 200–250 g, obtained from Charles River Canada, were individually housed in a climatically controlled colony room (21 ± 1°C) on a 12 L : 12 D cycle (light 0600–1800 h). Food and water were available continuously in the home cages.

Surgical Procedures

All rats were anaesthetized with sodium pentobarbital (Somnotol) (60 mg/kg, IP). The animals were prepared with chronic indwelling stainless steel guide cannulae (0.64 mm diam.) stereotaxically implanted in the dorsal striatum at the coordinates: 0.26 mm posterior to bregma, 3.0 mm lateral to the midline, and 3.4–4.0 mm ventral to the dura mater, with the incisor bar set 3.3 mm below the horizontal plane passing through the interaural line (25). Guide cannulae were positioned 1.0–2.0 mm dorsal to the target site as injection cannulae extended 1.0–2.0 mm beyond the guide. Cannulae were anchored to the skull with stainless steel screws and acrylic cement. The cannulae were sealed between injections with stainless steel obturator pins. The number of rats cannulated for Experiments 1, 2, 3, and 4 was 30, 18, 19, and 18, respectively. The side of cannulation for all experiments was balanced with an equal number of animals being implanted in the right and left striatum, except in Experiment 3 where nine animals were implanted in the left striatum and 10 in the right.

Apparatus

Three polyurethane-sealed circular wooden bases, 30 cm in diameter, enclosed within a cylinder of wire mesh 30 cm high, were fitted with Plexiglas covers.

Drugs

Porcine NPY (Peninsula Laboratories, Belmont, CA) was dissolved in 0.9% saline vehicle in double distilled water. (+)-Amphetamine (Smith, Kline and French, Canada), *trans*-flupenthixol, and *cis*-flupenthixol (H. Lundbeck, Denmark) were dissolved in 0.9% saline vehicle. All drugs were dissolved daily, prior to behavioral testing.

Central Injections

Central microinjections of the drug or the vehicle were delivered in a volume of 0.5 μl using a 10-μl Hamilton microsyringe. Injection cannulae were constructed with 0.31-mm diameter stainless steel tubing cut to extend 1.0–2.0 mm beyond the tip of the guide cannula and attached to the microsyringe by a length of polyethylene tubing. The injection was delivered in 30 s and the injection cannula was left in place for an additional 30 s to ensure sufficient diffusion and avoid withdrawing of the drug during removal of the injection cannulae. The obturator pin was then reinserted into the guide cannula.

General Procedure

The four experiments followed the general procedures outlined below. Specific details of each experiment are noted in the separate experimental sections that follow.

Testing began approximately 7 days after surgery. Each animal was tested seven times as follows: 1) no central injection; 2) central injection of the vehicle; 3–5) each of the three drug doses with order of administration counterbalanced across rats over three sessions; 6) replication of vehicle; 7) replication of no central injection. Three days were allowed between test sessions for each animal. A test session began with the intrastriatal injection and placement in the circular arena. All animals were tested under reduced lighting (60 W).

All complete turns (360°) ipsilateral and contralateral to the side of the cannula, were counted during each observation period. Three animals were scored during each 20-min session, observation periods being at 0–5 and 15–20 min. The clock was stopped during the time taken to administer the central injections (maximum of 4 min). Animals were started at staggered intervals of 5 min such that only one animal was being scored at any time. Therefore, each animal was scored for a total of 10 min, in two 5-min blocks at approximately equal intervals throughout the 20-min session. Animals were tested at approximately the same time each test day, between 0730–1300 h.

Circling behavior was expressed as the ratio of ipsilateral turns to the total number of turns (ipsilateral + contralateral). Ratio values of 0.5 indicated equal circling in both directions. Values less than 0.5 indicated a tendency for contralateral circling (away from the side of the central injection) whereas values greater than 0.5 indicated a tendency for ipsilateral circling (towards the side of the central injection).

Histology

At the conclusion of behavioral testing, animals were sacrificed for histological confirmation of cannula placements. Rats were injected with an overdose of sodium pentobarbital, exsanguinated, and perfused intracardially with 4% formalin. Frozen coronal sections (50 μm) were mounted and stained with thionin.

Experiment 1

Thirty animals were implanted with cannulae. Amphetamine sulphate was administered in doses of 6, 12, and 25 μg/0.5 μl.

Experiment 2

Eighteen animals were implanted with cannulae. Each animal was tested seven times as follows: 1) no central injection;

2) central injection of *cis*-flupenthixol (20 μg/0.5 μl) 15 min prior to central injection of amphetamine's vehicle; 3) central injection of *trans*-flupenthixol (20 μg/0.5 μl), the inactive geometric isomer of *cis*-flupenthixol, 15 min prior to central injection of amphetamine (25 μg/0.5 μl); 4) central injection of *cis*-flupenthixol (20 μg/0.5 μl) 15 min prior to central injection of amphetamine (25 μg/0.5 μl); 5) replication of the third session; 6) replication of the second session; 7) replication of no central injection.

Experiment 3

Nineteen animals were implanted with cannulae. The three doses of NPY were 0.01, 0.1, and 1.0 μg/0.5 μl.

Experiment 4

Eighteen animals were implanted with cannulae. Each animal was tested seven times as follows: 1) no central injection; 2) central injection of *cis*-flupenthixol (25 μg/0.5 μl) 15 min prior to central injection of NPY's vehicle; 3) central injection of *trans*-flupenthixol (25 μg/0.5 μl) 15 min prior to central injection of NPY (0.1 μg/0.5 μl); 4) central injection of *cis*-flupenthixol (25 μg/0.5 μl) 15 min prior to central injection of NPY (0.1 μg/0.5 μl); 5) replication of the third session; 6) replication of the second session; 7) replication of no central injection. The dose of flupenthixol (25 μg/0.5 μl) used here was slightly higher than that used in Experiment 2 (20 μg/0.5 μl) because that dose, although significantly reducing the turning effect of amphetamine, did not completely block it.

RESULTS

Histology

A total of 85 animals were implanted with cannulae; 41 were verified as accurate cannulae placements. A verified accurate placement was defined as a cannula tip located −0.92 to 1.00 mm of bregma, 2.5 to 4.0 mm lateral to the midline, and 3.0 to 6.0 mm ventral to the dura mater (25). The remaining animals were discarded due to defective cannulae (n = 16) or inaccurate placements (n = 28). Many of the inaccurate placements were from Experiment 1, where cannulae tips were frequently dorsal to the striatum. The number of animals included in the statistical analyses for the accurate placements for the four experiments were 10, 8, 11, and 12, respectively. The actual cannulae placements for all animals that completed testing are illustrated in Fig. 1.

No Injection and Saline Circling Scores

Initially, two two-factor repeated-measures analyses of variance (ANOVAs) were performed, one for the first and second no-injection sessions and one for the first and second saline or *cis*-flupenthixol and saline sessions. The variables were sessions and time. In all four experiments neither the main effect of sessions nor the interactions were significant for the no-injection or saline scores and thus these scores were combined for further analyses. The results for each experiment were then subjected to ANOVA using the Geiser-Greenhouse adjusted degrees of freedom to reduce possible error associated with the use of repeated measures. Individual comparisons for each dependent variable were made with post hoc tests (Dunnett's t) when warranted.

Turning Frequency

Circling ratios were calculated as number of ipsilateral turns over total turns. The (mean ± SEM) total turns per 5 min for sessions 1 and 7, 2 and 6, and 3, 4, and 5 from each experiment, averaged over the two observation periods, are shown in Table 1. Values range from 3.1 to 5.4 turns per 5 min. ANOVA of turning frequency from each experiment yielded significant effects of time in every case, but the treatments effect only achieved significance for Experiment 2, $F(2.24, 15.65) = 2.91$, $p < 0.01$. This reflected decreased turning frequency during sessions 2 and 6 when *cis*-flupenthixol was injected alone. The significant time effects indicate large decreases in total turns from a range of 3.7–7.8 turns per 5 min during the first observation period to a range of 1.5–5.0 during the second.

Experiment 1

The no-injection and saline treatments produced circling ratios of approximately 0.5, indicating no directional bias. The 6-, 12-, and 25-μg amphetamine doses produced progressively lower circling ratios, indicating a contralateral bias (Fig. 2A). The ANOVA from the combined no-injection, combined saline, and the three amphetamine sessions confirmed a significant treatment effect, $F(2.72, 24.49) = 4.81$, $p < 0.01$, and nonsignificant time, $F(1, 9) = 1.84$, $p > 0.05$, and interaction effects, $F(2.85, 25.67) = 2.50$, $p > 0.05$. Post hoc pairwise comparisons between each treatment and saline revealed that the 25-μg amphetamine circling ratio was significantly lower than saline.

It is noteworthy that, of the animals that completed testing, the discarded animals (n = 11) with placements dorsal to the target area (Fig. 1) failed to circle in response to amphetamine. Their means ± SEM, averaged over the two observation periods, for no-injection, saline, and 6, 12, and 25 μg of amphetamine were 0.49 ± 0.06, 0.54 ± 0.07, 0.35 ± 0.10, 0.48 ± 0.07, and 0.40 ± 0.08, respectively. ANOVA revealed no significant effects.

Experiment 2

Initial ANOVAs were performed on the circling ratio scores: one for the two no-injection sessions, one for the two *cis*-flupenthixol + saline sessions, and one for the two *trans*-flupenthixol + amphetamine sessions. Results revealed no significant differences. Therefore, every animal's scores from each of the replicated treatments were combined for subsequent analyses.

The no-injection and *cis*-flupenthixol + saline treatments produced circling ratios slightly greater than 0.5, indicating a small bias for ipsilateral circling. The *trans*-flupenthixol + amphetamine treatment produced a markedly lower circling ratio, indicating a contralateral bias, and cotreatment with *cis*-flupenthixol + amphetamine partially reversed this effect (Fig. 2B). The ANOVA performed on the ratio scores from the combined no-injection, *cis*-flupenthixol + saline, and *trans*-flupenthixol + amphetamine sessions, and the *cis*-flupenthixol + amphetamine session revealed a significant treatment effect, $F(1.82, 12.71) = 6.77$, $p < 0.01$, and nonsignificant time, $F(1, 7) = 0.67$, $p > 0.05$, and interaction effects, $F(2.26, 15.81) = 0.79$, $p > 0.05$. Post hoc pairwise comparisons between each treatment and *cis*-flupenthixol + saline revealed that the *trans*-flupenthixol + amphetamine circling ratio was significantly lower.

Experiment 3

As shown in Fig. 2C, the no-injection and saline treatments produced circling ratios of approximately 0.5, indicating no

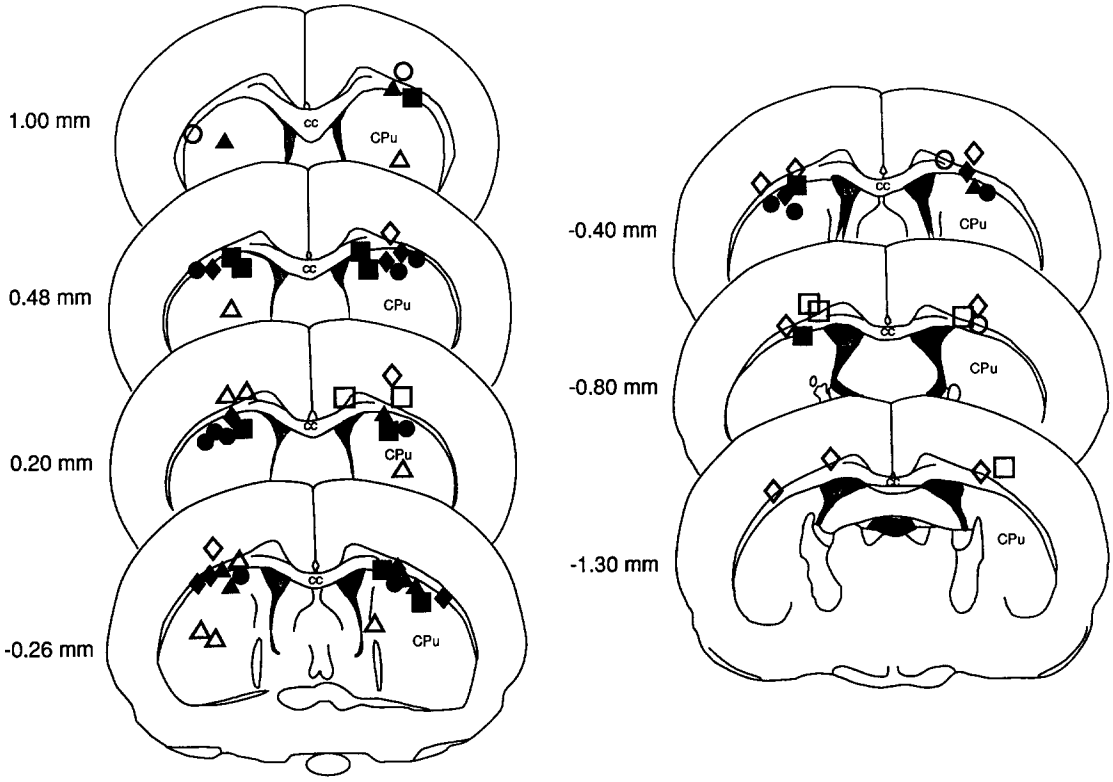

FIG. 1. Cannula placements for animals classified as hits (filled symbols) or misses (open symbols) in experiments 1 (♦), 2 (▲), 3 (■), and 4 (●). Coronal sections were reproduced from (25) and the distance anterior to bregma is indicated to the left of each section. Of the 30, 18, 19, and 18 rats from Experiments 1–4, respectively, 21, 17, 17, and 16 are shown. Remaining rats never completed testing due to defective cannulae.

directional bias. NPY produced lower circling ratios, especially at the 0.10 μg/0.5 μl dose, indicating a contralateral bias. The ANOVA confirmed a significant treatment effect, $F(2.79, 27.91) = 3.35$, $p < 0.05$, and nonsignificant time, $F(1,10) = 0.01$, $p > 0.05$, and interaction effects, $F(2.91, 29.14) = 0.77$, $p > 0.05$. Post hoc pairwise comparisons between each treatment and saline revealed that the 0.1-μg NPY circling ratio was significantly lower than saline.

TABLE 1

MEAN ± SEM TOTAL NUMBER OF TURNS PER 5 MIN FOR EACH EXPERIMENT

		Session				
Exp.	(*N*)	1 + 7	2 + 6	3(+5)	4	5
1	(10)	3.7 ± 0.6	3.7 ± 0.5	3.1 ± 0.5	3.8 ± 0.5	3.5 ± 0.5
2	(8)	5.2 ± 0.6	4.4 ± 0.7*	5.3 ± 0.8	5.4 ± 0.8	
3	(11)	3.4 ± 0.4	3.4 ± 0.4	3.6 ± 0.5	3.5 ± 0.6	3.2 ± 0.5
4	(12)	4.9 ± 0.5	4.9 ± 0.5	4.7 ± 0.6	3.9 ± 0.6	

Sessions 1 and 7 were always no injection. Sessions 2 and 6 involved saline injections in Experiments 1 and 3; in Experiments 2 and 4 these sessions were preceded 15 min earlier by injections of *cis*-flupenthixol (20.0 and 25.0 μg, respectively) and immediately by injections of saline. Sessions 3, 4, and 5 in Experiments 1 and 3 were preceded by injections of amphetamine (6, 12, and 25 μg) or NPY (0.01, 0.1, and 1.0 μg), respectively. Sessions 3 and 5 in Experiments 2 and 4 were preceded 15 min earlier by injections of *trans*-flupenthixol (20.0 and 25.0 μg, respectively) and immediately by injections of amphetamine (25 μg) or NPY (0.1 μg), respectively. Session 4 in Experiments 2 and 4 was preceded 15 min earlier by injections of *cis*-flupenthixol (20.0 and 25.0 μg, respectively) and immediately by injections of amphetamine (25 μg) or NPY (0.1 μg), respectively. All injection volumes were 0.5 μl.

*There was a significant decrease in total turns during these *cis*-flupenthixol followed by saline sessions.

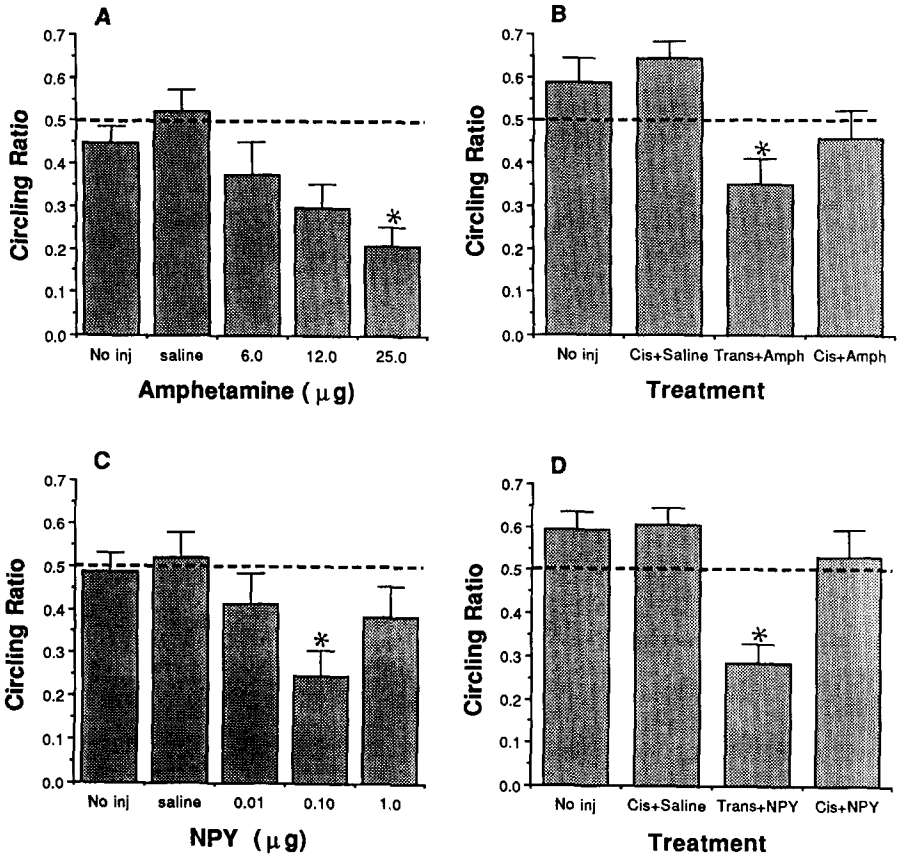

FIG. 2. Mean ± SEM circling ratios for all experiments. (A) Mean ratios for the combined no-injection (No inj) sessions, combined saline sessions, and 6.0-, 12.0-, and 25.0-μg (in 0.5 μl) doses of amphetamine. (B) Mean ratios for the combined no-injection (No inj) sessions, combined *cis*-flupenthixol (20 μg/0.5 μl) + saline (0.5 μl) sessions (Cis + Saline), combined *trans*-flupenthixol (20 μg/0.5 μl) + amphetamine (25 μg/0.5 μl) sessions (Trans + Amph), and for the *cis*-flupenthixol (20 μg/0.5 μl) + amphetamine (25 μg/0.5 μl) session (Cis + Amph). (C) Mean ratios for the combined no-injection (No inj) sessions, combined saline sessions and 0.01-, 0.1-, and 1.0-μg (in 0.5 μl) doses of NPY. (D) Mean ratios for the combined no-injection (No inj) sessions, combined *cis*-flupenthixol (25 μg/0.5 μl) + saline (0.5 μl) sessions (Cis + Saline), combined *trans*-flupenthixol (25 μg/0.5 μl) + NPY (0.1 μg/0.5 μg) sessions (Trans + NPY), and for the *cis*-flupenthixol (25 μg/0.5 μl) + NPY (0.1 μg/0.5 μl) session (Cis + NPY). Asterisks indicate ratios significantly different from the saline or Cis + Saline treatment determined by post hoc Dunnett's tests following observation of a significant treatment effect in each experiment.

Experiment 4

As in Experiment 2, initial ANOVAs were performed on the circling ratio scores for the two no-injection sessions, the two *cis*-flupenthixol + saline sessions, and the two *trans*-flupenthixol + NPY sessions. Results revealed no significant differences and resulted in every animal's scores from each of the replications being combined for subsequent analyses.

The no-injection and *cis*-flupenthixol + saline treatments produced circling ratios slightly greater than 0.5, indicating a small ipsilateral bias. The *trans*-flupenthixol + NPY treatment produced a markedly lower circling ratio, indicating a contralateral bias, and cotreatment with *cis*-flupenthixol + NPY reversed this effect (Fig. 2D). The ANOVA performed on the ratio scores from the combined no-injection, *cis*-flupenthixol + saline, and *trans*-flupenthixol + NPY sessions, and the *cis*-flupenthixol + NPY session revealed nonsignificant time, $F(1, 11) = 0.85, p > 0.05$, and interaction effects, $F(2.18, 24.02) = 1.18, p > 0.05$, but a significant treatment effect, $F(2.82, 31.01) = 9.76, p < 0.005$. Post hoc pairwise comparisons revealed that the *trans*-flupenthixol + NPY circling ratio was significantly lower than the *cis*-flupenthixol + saline treatment.

In summary, both amphetamine and NPY injected unilaterally into the striatum produced contralateral circling behavior. This directional bias was antagonized by *cis*-flupenthixol, a DA antagonist, in the case of amphetamine, and fully blocked in the case of NPY.

DISCUSSION

Control Procedures for the Circling Paradigm

It was found that during the first and second no-injection test sessions for each of the four experiments mean ratios were approximately 0.5 and did not differ significantly. This result

is in agreement with previous studies from this laboratory (5,18,22,23,32) and provides further evidence that neither chronic cannulation nor the series of five central injections had enduring effects on the directional bias shown by the rats.

The first and second saline test sessions for Experiments 1 and 3 yielded mean ratios of approximately 0.5. This finding demonstrated that circling did not result simply from the mechanical effects of injecting a small volume of fluid into the striatum of a rat and is consistent with previous findings from this laboratory (5,18,22,23,32). Furthermore, circling following amphetamine or NPY cannot be attributed to a sensitization to the effects of repeated injections because the pre- and postdrug saline ratios did not differ significantly.

The first and second *cis*-flupenthixol + saline test sessions for Experiments 2 and 4 also yielded mean ratios of approximately 0.5. Besides supporting the contention that circling cannot be attributed to mechanical effects of intrastriatal injections, these results also show that the DA antagonist *cis*-flupenthixol fails to induce turning in otherwise intact rats. This finding is in agreement with the results of Costall et al. (7) and with previous results from this laboratory (18).

Further support for the contention that the results are not due to repeated injections to the same animal comes in two forms. Firstly, there was no significant difference between the first and second *trans*-flupenthixol + amphetamine sessions for Experiment 2 (mean ratios being approximately 0.35); similarly, there was no significant different between the first and second *trans*-flupenthixol + NPY sessions for Experiment 4 (mean ratios being approximately 0.30). Secondly, there is the finding that amphetamine produced contralateral circling in a dose-dependent manner even though the doses were administered in a counterbalanced order across rats.

The blockade of amphetamine- and NPY-produced turning by *cis*-flupenthixol in Experiments 2 and 4, respectively, cannot be attributed to nonpharmacological properties of *cis*-flupenthixol. Thus, the inactive geometric isomer, *trans*-flupenthixol, failed to alter amphetamine- or NPY-produced turning in sessions 3 or 5 of Experiments 2 and 4, respectively. These results support the contention that the effects of *cis*-flupenthixol are related to its properties as a DA receptor antagonist.

The possibility that the present results were due to diffusion of the drug to the nucleus accumbens seems unlikely. It has been shown that a 1.0-μl injection volume diffuses into a sphere of approximately 1.0-mm diameter (24), and an even smaller volume (0.5 μl) was used here. Furthermore, if the drug effect was due to diffusion to a remote site, a delay in its onset of action would be expected. In the present study, the maximal effects on turning were seen in the first 5-min observation period, making a remote site of action less likely.

In this and in previous studies from this laboratory (18), it has been found that turning does not occur if cannulae tips are in or dorsal to the corpus collosum dorsal to the striatal target site. However, as shown in Fig. 1, turning is seen with a variety of placements within the dorsal anterior portion of the striatum. Whether placements in other regions of the striatum can produce similar effects remains to be investigated.

In each experiment, directionality and total turns were evaluated. However, it appears that only directionality was influenced in a consistent manner. Total turns showed no reliable changes following amphetamine or NPY treatments either alone or in combination with flupenthixol. Only *cis*-flupenthixol alone in Experiment 2, but not the same treatment in Experiment 4, reliably decreased turning frequency. The finding of nonsignificant effects of centrally infused pharmacological compounds on total turns is in accord with previous studies from this laboratory as is the finding of consistent effects on the directional bias of the animal (18). These findings underscore the utility of directional bias or relative turning as a sensitive, dependent measure reflecting bilateral differences in striatal function.

The control procedures for the behavioral experiments reveal that the circling ratio results of the four experiments were highly reliable. Results probably were not due to repeated injections, mechanical effects of the injection per se, or diffusion of the drug away from the dorsal striatum.

GENERAL DISCUSSION

In Experiment 1, it was found that amphetamine injected unilaterally into the dorsal striatum produced contralateral circling in a dose-dependent manner. This is in agreement with the findings of Costall and Naylor (8), who reported that animals pretreated with a neuroleptic and a monoamine oxidase inhibitor circled contralaterally when administered amphetamine (in doses of 50 and 100 μg/μl) into the caudate nucleus.

In vivo techniques, such as microdialysis, which directly measure the action of drugs on DA activity, have shown that amphetamine, when injected into the striatum, increases DA release while decreasing DA metabolite levels (21,38,39). Similar results have been reported for amphetamine administered systemically (6,20,30,39). Thus, the use of the circling paradigm and the inference that it provides a reliable index of DA activity is supported by independent biochemical studies.

To further assess the involvement of DA in amphetamine-induced contralateral circling behavior, Experiment 2 attempted to block amphetamine-induced circling with *cis*-flupenthixol, a DA antagonist. As in the first experiment, intrastriatal amphetamine (25 μg) produced contralateral circling and *cis*-flupenthixol partially blocked this effect. Thus, treatment with microinjections of amphetamine plus the DA antagonist did not lead to circling ratios significantly different from those seen for *cis*-flupenthixol alone.

The observation that unilateral intrastriatal injections of NPY produced contralateral circling has not been reported previously. An NPY dose of 0.10 μg reliably produced contralateral turning in Experiments 3 and 4. A dose of 0.01 μg was ineffective, showing that the NPY effect depended on dose. The failure of 1.0 μg to produce a significant effect might suggest that NPY-produced turning is only seen at moderate doses. Further studies are needed to explore fully the relationship between NPY dose and circling ratio.

NPY-mediated circling appeared to depend on striatal DA. Thus, injections of the DA antagonist *cis*-flupenthixol, but not its inactive geometric isomer *trans*-flupenthixol, blocked the NPY effect. It is noteworthy that *cis*-flupenthixol, when injected alone, failed to produce ipsilateral circling, making the possibility that the two drugs simply had opposite effects that cancelled each other out seem unlikely. In neurochemical experiments, intrastriatal NPY has been reported to produce increases in striatal DA release (3). This and the present behavioral results suggest that NPY in the striatum acts to release DA that has a behaviorally relevant effect.

NPY produced circling in the same contralateral direction as the DA-releasing agent amphetamine. There is a relationship between the effects of unilateral and bilateral injections of DA agents into the striatum. Thus, drugs that produce contralateral circling when injected unilaterally typically pro-

duce increased activity when injected bilaterally, and drugs that produce ipsilateral circling when injected unilaterally produce decreased activity when injected bilaterally. It is well documented that bilateral increases in striatal DA lead to increases in locomotor behavior (4). From this point of view, the finding that intrastriatal NPY produced contralateral circling in the present study also is consistent with a DA-like stimulant action.

Previous researchers have found that ICV NPY leads to decreases in locomotor activity (14,16,17). As previous experiments have shown that ICV NPY stimulated the synthesis and release of DA in the striatum, these behavioral observations may seem contradictory. One explanation may be that the site of action of NPY that is relevant for its behavioral effects following ICV administration is not the striatum. Thus, NPY has been shown to be widely distributed in the brain (10). It will be the task of future studies to identify the site(s) of action of ICV NPY that are responsible for its motor-suppressant effects.

In conclusion, the present experiments have shown that unilateral intrastriatal injections of NPY, like amphetamine, lead to contralateral circling that is blocked by coadministration of the DA receptor blocker, *cis*-flupenthixol. These results suggest that NPY in the striatum modulates DA release and that this can influence ongoing behavioral activity.

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