Pharmacology Biochemistry and Behavior, Vol. 65, No. 2, pp. 203–207, 2000

0091-3057/00/$–see front matter

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PII S0091-3057(99)00194-X

Turning in Rats Following Intraaccumbens Shell Injections of Amphetamine or Eticlopride

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Received 13 November 1998; Revised 30 April 1999; Accepted 4 June 1999

BERNSTEIN, S. N. AND R. J. BENINGER. *Turning in rats following intraaccumbens shell injections of amphetamine or eticlopride.* PHARMACOL BIOCHEM BEHAV **65**(2) 203–207, 2000.—In recent years there has been interest in possible differential behavioral functions of the core and shell subregions of the nucleus accumbens. The present study compared the effects of accumbens core and shell injections of (+)-amphetamine on turning behavior in rats; the turning effects of the selective D_2 antagonist (−)-eticlopride injected into the shell were also tested. Rats (n = 28) were implanted unilaterally with guide cannulae and subsequently tested for turning in seven 20-min sessions. Amphetamine (10.0 and 20.0 μg but not 5.0 μg/0.5 μl) elicited contralateral turning following injection into the shell, while the same doses into the core had no effect. In animals given systemic amphetamine (2.0 mg/kg), eticlopride (10.0 but not 1.0 or 0.1 μg/0.5 μl) injected into the shell region of the accumbens produced ipsilateral turning. These results suggest that the accumbens shell, but not the core, is a critical site for turning behavior. © 2000 Elsevier Science Inc.

Accumbens shell Accumbens core Turning behavior D_2 receptors Eticlopride Amphetamine

A wide range of evidence shows that unconditioned behaviors are mediated by dopaminergic neurotransmission [see (1) for review]. One approach is to create a dopamine (DA) imbalance on the two sides of the brain, leading to turning behavior (13). In general, drugs that produce locomotor stimulation when injected systemically or bilaterally into certain regions of the brain produce contralateral rotation when injected unilaterally. Conversely, drugs that produce locomotor attenuation following systemic or bilateral central injection generally produce ipsilateral rotation when given unilaterally (5,6).

Dose-dependent contralateral circling has been demonstrated following unilateral intra-accumbens (NAc) injections of amphetamine (2). Following the distinction between core and shell subregions of the NAc (4,14), it was demonstrated further that contralateral turning could be induced by a combination of agents acting at different DA receptor subtypes in the shell, but not the core of this nucleus (7). These results suggest that the effects of intra-NAc amphetamine on turning behavior are due to the action of amphetamine in the shell.

The present study examined the hypothesis that amphetamine injected unilaterally into the NAc shell but not the core would produce contralateral turning. Results supported this hypothesis. An additional study evaluated the effects of unilateral intra-NAc shell injections of the DA D_2 receptor antagonist eticlopride on turning in animals pretreated with amphetamine systemically. It was hypothesized that this group would show ipsilateral turning, confirming that turning in either direction can be elicited by DA agents in the shell of the NAc.

METHOD

All procedures were in accordance with the Animals for Research Act, the Guidelines of the Canadian Council on Animal Care, and were approved by the Queen's University Animal Care Committee.

Subjects

Experimentally naive male Wistar rats (Charles River, Canada) weighing 200–250 g were housed in pairs in a temperature-controlled (21°C) environment on a 12-h light–dark cycle (lights on at 0700 h). The animals were given free access to food and water in the home cage, and were handled daily.

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Surgeries

Rats were anesthetized with halothane (1.5–4%) and stereotaxically implanted unilaterally with a stainless steel guide cannula (0.6-mm diameter) aimed at the shell or core of the NAc (anterior = 1.7 mm, ventral = 6.5 or 6.0 mm, lateral = 1.0 or 2.0 mm, respectively, from bregma) according to Paxinos and Watson (9). In half of the rats cannulae were placed on the right side, and in the other half the left. The guide cannulae were affixed to the skulls with dental acrylic and four skull screws. For attachment to the rotomoter, an arborite chip was fixed in the acrylic. Immediately following surgery, rats received either buprenorphine (0.01 mg/kg) or banamine (5.0 mg/kg) subcutaneously as an analgesic, and six separate subcutaneous injections around the wound comprising approximately 0.1 cc of Marcaine as a local anaesthetic. Polysporin and wound powder were applied to the affected area. Systemic analgesics also were given 24 h after surgery. Rats were given 7 days to recover before the experimental protocol began.

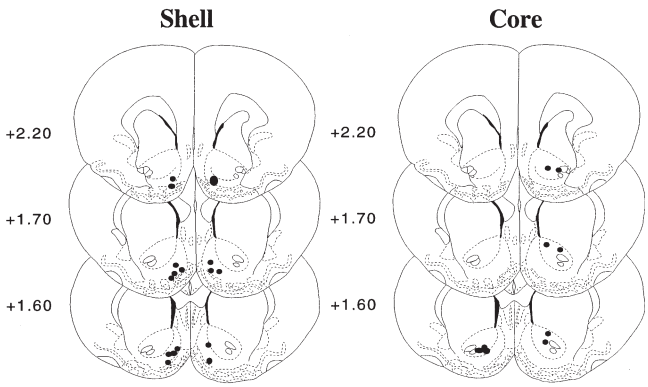

FIG. 1. Location of cannulae placements: coronal sections depicting cannulae placements (n = 28) in the shell (left) and the core (right) of the nucleus accumbens. Placements are shown on representative sections according to the atlas in (9). Coordinates are the anterior distance (mm) from bregma.

Drugs

Drugs were prepared anew each day before experimental testing was begun. (+)-Amphetamine sulphate (SmithKline Beecham Pharma Inc. Canada), (−)-eticlopride hydrochloride and (+)-eticlopride hydrochloride [Research Biochemicals International, Natic, MA] were dissolved in saline (0.9% NaCl).

Central Injections

Injections of 0.5 μl of fluid were made through polyethylene tubing attached to a stainless steel cannula (0.3-mm diameter) extending 1.0 mm below the guide (ventral = 7.5 or 7.0 mm). Injections were administered over a 30-s period using a microinfusion pump attached to a 10-μl Hamilton syringe, after which the cannula was left in place for an additional 45 s to allow for diffusion. Behavioral testing began immediately following injections.

Apparatus

Rats were tested in circular chambers (30-cm diameter) consisting of a urethane-sealed wooden base, wire mesh sides (30 cm high), and a clear Plexiglas cover. The rats were tested separately, and their circling behavior (including the number of turns and the direction of turning) recorded by the experimenter either visually (amphetamine in the NAc shell experiment), or via an automated rotometer. Previous studies have shown that these approaches yield similar results (11). The rotometer apparatus consisted of a notched rotating disk triggering four photoelectric cells located at 90° intervals. A telescoping metal rod, attached at one end to the disk and at the other end to the arborite chip embedded in the skull cap allowed freedom of movement. The number of 360° in each direction was recorded on a single board computer and relayed to a Macintosh computer for subsequent analyses.

Procedure

Three experiments were carried out. Each consisted of seven 20-min test sessions that took place 48 h apart. Sessions occurred between 1900 and 2400 h, during the beginning of the dark period of the light–dark cycle. In each experiment, the first and last session was preceded by no injection.

Two experiments evaluated the effects of amphetamine (5.0, 10.0, 20.0 μg), one in the shell of the NAc, and the other

TABLE 1

MEAN (± SEM) TURNING RATIOS AND TOTAL TURNS FOR SESSIONS 1, 2, 6, AND 7 FOR EACH EXPERIMENT

		Session			
		No Injection		Vehicle*	
Experiment	n	1	7	2	6
		Turning ratios			
AMPH shell	9	0.48 ± 0.07	0.54 ± 0.05	0.52 ± 0.06	0.56 ± 0.08
AMPH core	10	0.34 ± 0.07	0.44 ± 0.06	0.40 ± 0.06	0.44 ± 0.09
Eticlopride	9	0.41 ± 0.06	0.59 ± 0.06	0.52 ± 0.07	0.67 ± 0.08
		Total turns			
AMPH shell	9	7.1 ± 1.1	5.5 ± 1.2	6.4 ± 1.4	7.6 ± 1.4
AMPH core	10	14.7 ± 1.8	17.5 ± 2.4	21.6 ± 2.4	23.7 ± 3.2
Ethiclopride	9	18.2 ± 2.4	18.1 ± 2.4	31.6 ± 4.1	24.8 ± 3.5

*Eticlopride sessions 2 and 6 were preceded by injections of amphetamine.
Abbreviaitons: AMPH = amphetamine.

the same doses in the core. Sessions 2 and 6 were preceded by an injection of saline vehicle (0.5 μl), and the three doses of amphetamine were given in different orders to different rats, randomizing any possible effects of order over sessions 3, 4, and 5. Treatments were given immediately before placement in the test apparatus. For the NAc shell experiment, turning behavior (number of 360° turns in each direction) was assessed visually; a second rater independently evaluated the animals in some of the sessions.

The third experiment tested the effects of eticlopride. Prior to sessions 2 and 6, the less active enantiomer (+)-eticlopride (10 μg) was given and prior to sessions 3, 4, and 5 the active enantiomer (−)-eticlopride (0.1, 1.0, 10 μg) was given, the order of the doses being counterbalanced across rats. Systemic amphetamine (2.0 mg/kg IP) preceded each session by 15 min.

Histology

Upon completion of behavioral testing, all animals were sacrificed through inhalation of CO_2. Brains were extracted and stored in a 10% formalin solution for at least 1 week, after which they were sectioned (60 μm) on a freezing stage microtome, mounted, and stained with thionine. The locations of the cannulae tracts were determined by an observer blind to the behavioral results.

Statistics

Circling behavior was calibrated as a ratio of the number of full turns (360°) made ipsilateral to the injection side divided by the total number (ipsilateral plus contralateral) of full turns. A constant of 0.01 was added to each value, so that if no turns were performed during a session the resulting ratio would be 0.5. Accordingly, a ratio of 0.5 would indicate a nondirectionally biased session and a lower or higher ratio would indicate contralateral or ipsilateral turning, receptively (12). The total number of full (360°) turns over a session was analyzed as a measure of overall locomotor activity.

A correlated *t*-test was performed on the data from the first and last no injection sessions. If no significant difference was found, the data were pooled before the analysis of variance (ANOVA) was carried out. Similarly, the two vehicle [or (+)-eticlopride] sessions were compared using a correlated *t*-test, and again if no significant difference was found the data were pooled before the ANOVA was carried out.

For each experiment, the combined no-injection, combined vehicle [or (+)-eticlopride], and each drug dose were subjected to a one-way repeated measures ANOVA for both dependent variables: turning ratio, and total turns. If the ANOVA revealed a significant treatment effect, Dunnet's *t*-tests were used as post hoc tests of drug effect, comparing vehicle controls [or (+)-eticlopride] with the individual doses of drug to determine the source of differences.

The amphetamine in the NAc shell experiment was carried out using visual assessment of turning instead of the rotometer. In some of the sessions, an independent second observer blind to the treatment condition scored the rats to allow for an assessment of interrater reliability. Interrater reliabilities were analyzed using a Pearson's product-moment correlation between turning ratios recorded by the experimenter and those recorded by the second observer.

RESULTS

Figure 1 gives an overview of the shell regions in which the injection sites were located ($n = 19$); rats with injection sites outside this region were discarded before overall analyses were carried out. Also shown are the locations of the guide cannulae in the NAc core ($n = 10$). Tissue damage generally was limited to the region of the cannula tract and injector tip.

Two raters independently scored some of the observation periods in the amphetamine in the NAc shell experiment. A Pearson's product-moment correlation evaluating reliability between the two behavioral observers using 60 paired 2-min observations revealed a coefficient of 0.93, which is considered highly reliable.

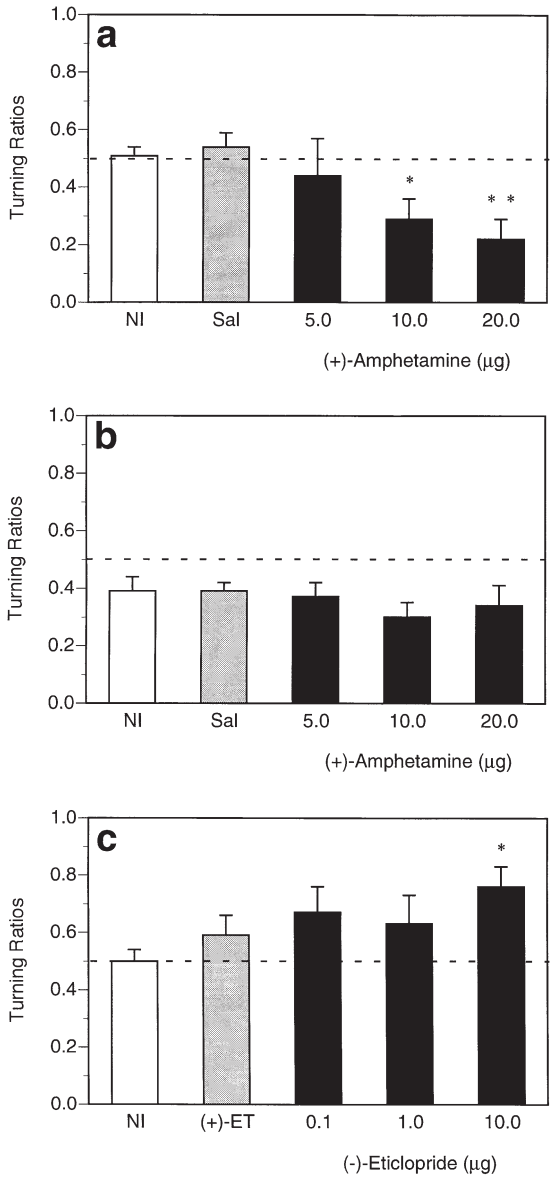

FIG. 2. Dose effects of intra-accumbens injections of dopamine agonists and antagonists on rotation. (a) Amphetamine injections into the accumbens shell caused contralateral rotation. (b) Amphetamine injections into the accumbens core failed to elicit turning. (c) (−)-Eticlopride into the shell combined with systemic amphetamine-elicited ipsilateral turning. Dashed lines indicate an unbiased turning ratio of 0.5. NI = No Injection; Sal = Saline; ET = eticlopride. $*p < 0.05$, $**p < 0.01$, in Dunnett's *t*-test compared to sal or (+)-ET following significant treatment effect in ANOVA.

For both dependent variables, no differences were found between no injection for session 1 vs. 7 nor for vehicle [or (+)-eticlopride] scores for sessions 2 vs. 5 (Table 1). Thus, the data from the pair of sessions entering into each comparison were averaged for each rat.

Turning Ratios

Generally, no injection or vehicle [or (+)-eticlopride] injections produced turning ratios with a value of about 0.5, indicating no directional bias (Fig. 2). Injections of amphetamine into the core of the NAc appeared to produce little change in directional bias at any dose compared to no injection or vehicle. Amphetamine or (−)-eticlopride into the shell of the NAc, on the other hand, led to dose-dependent contra- or ipsilateral turning, respectively. [Note that (−)-eticlopride-treated animals also received systemic amphetamine.]

This description of the turning ratios is supported by the results of statistical analyses. Thus, the one-way ANOVAs of treatments for the amphetamine core experiment yielded nonsignificant results. On the other hand, the corresponding ANOVAs for the amphetamine shell or eticlopride experiments yielded significant effects, $F(4, 32) = 3.58$, $p < 0.05$, and $F(4, 32) = 3.14$, $p < 0.05$, respectively. Dunnett's tests for the amphetamine shell treatments comparing each drug dose to vehicle revealed significant contralateral turning following 10.0 or 20.0 μg/0.5 μl. Similar tests for the eticlopride experiment comparing treatments with the active enantiomer to the less active enantiomer revealed significant ipsilateral turning following 10.0 μg/0.5 μl of (−)-eticlopride.

Full Turns

Injections of amphetamine or (−)-eticlopride into the shell of the NAc did not significantly increase the total number of turns in either direction (Table 2). Injections of amphetamine into the core of the NAc increased the number of total turns at 10.0 and 20.0 μg/0.5 μl, $F(4, 36) = 44.00$, $p < 0.01$.

DISCUSSION

This study examined the role of the NAc core and shell in amphetamine-induced turning behavior. Results showed that amphetamine was effective at eliciting contralateral turning following NAc shell, but not core, injections. Furthermore, injections of the D_2 antagonist (−)-eticlopride elicited an ipsilateral directional bias.

Each experiment included four control trials over the course of the test sessions. In the days preceding drug trials, rats underwent a no-injection trial followed 2 days later by a vehicle trial. Following drug trials, rats received a vehicle trial and then no injection. Saline was used for the amphetamine control sessions. For the (−)-eticlopride sessions, the less active isomer (+)-eticlopride (3) was used in place of vehicle. No significant differences were found between any of the before and after control trials. Thus, there was no enduring effect on turning bias of the series of central injections, nor was there a significant change in response to the mechanical or other effects of central fluid injection from before to after the series of three drug injections.

Our finding that amphetamine injections into the NAc shell but not the core led to contralateral turning is in agreement with the results of Koshikawa et al. (7). These authors showed that the combined administration of quinpirole and SKF 38393 elicited contralateral turning in the shell of the NAc, but not the core.

The present study demonstrated that unilateral injection of the D_2 antagonist (−)-eticlopride (10.0 μg) produced ipsilateral turning when combined with systemic amphetamine. This followed expectations that rats would turn towards the side of central injection following D_2 receptor blockade and systemic amphetamine. Previous research has indicated that unilateral NAc injections of the direct-acting D_1- and D_2-like antagonists SCH-23390 and metoclopramide, respectively, when combined with systemic amphetamine produce ipsilateral turning (8); this study confirms these results for another D_2 DA receptor antagonist. Thus, there is evidence for an instrumental role for D_1 and D_2 receptors in shell-mediated turning.

TABLE 2

EFFECTS OF DOPAMINERGIC DRUGS ON TOTAL, CONTRALATERAL, AND IPSILATERAL FULL TURNS (MEAN ± SEM) IN 20-MIN SESSIONS

Experiment	NI Mean	VEH Mean	Low Dose	Med. Dose	High Dose
AMPH shell					
Total	6.3 ± 1.3	7.0 ± 2.0	7.5 ± 2.2	9.6 ± 2.5	11.7 ± 3.4
Contra	3.5 ± 1.6	3.4 ± 1.4	3.4 ± 1.5	6.3 ± 1.8	8.2 ± 3.0
AMPH core					
Total*	16.1 ± 1.2	22.6 ± 2.4	21.8 ± 2.2	36.6 ± 3.1*	41.2 ± 3.8*
Contra	9.7 ± 0.9	12.6 ± 1.7	15.2 ± 2.1	21.3 ± 4.7	23.1 ± 5.0
	NI Mean	(+)ET Mean	Low Dose	Med. Dose	High Dose
(−)ET shell/AMPH					
Total	18.2 ± 2.1	28.2 ± 3.0	33.8 ± 4.4	34.9 ± 6.8	22.2 ± 5.4
Ipsi	9.2 ± 1.4	16.4 ± 3.1	21.3 ± 3.4	20.9 ± 5.6	17.9 ± 5.4

Abbreviations: NI: no injection; VEH: vehicle, contra: contralateral full turns; Ipsi: ipsilateral full turns; AMPH: amphetamine; ET: eticlopride.

Low, medium, high dose: AMPH (5.0, 10.0, 20.0 μg); ET (0.1, 1.0, 10.0 μg);

*$p < 0.01$ vs. VEH in Dunnett's tests following significant treatment effect in ANOVA

The analysis of total turns revealed a number of findings. In particular, it was observed that the NAc shell injections of amphetamine had no significant effect on total turns, whereas total turns increased with amphetamine doses in the core. This shows a dissociation between total turns and directional bias because it was NAc shell but not core injections of amphetamine that produced turning. A similar dissociation between total turns and directional bias has been seen in our previous studies [e.g., (10)].

In summary, intra-NAc shell but not core injection of amphetamine or (−)-eticlopride were effective in eliciting locomotor asymmetry. These results add to other recent findings identifying dissociable behavioral functions for the NAc core and shell.

ACKNOWLEDGEMENTS

This study was funded by a grant from the Natural Sciences and Engineering Research Council of Canada to R.J.B.

REFERENCES

1. Beninger, R. J.: The role of dopamine in locomotor activity and learning. Brain Res. Rev. 6:173–196; 1983.
2. Colle, L. M.; Wise, R. A.: Circling induced by intra-accumbens amphetamine injections. Psychopharmacology (Berlin) 105:157–161; 1991.
3. de Paulis, T.; Kumar, Y.; Johansson, L.; Ramsky, S.; Hall H.; Sallemark, M.; Angeby-Moller, K.; Ogren, S. O.: Potential neuroleptic agents. 4. Chemistry, behavioural pharmacology, and inhibition of [^{3}H]spiperone binding of 3, 5-disubstituted N-[(1-ethyl—2-pyrrolidinyl)methyl]-6-methoxysalicylamides. J. Med. Chem. 29:61–69; 1986.
4. Heimer, L.; Zahm, D. S.; Churchill, L.; Kalivas, P. W.; Wohltmann, C.: Specificity in the projection patterns of accumbal core and shell in the rat. Neuroscience 41:89–125; 1991.
5. Holmes, L. J.; Bozarth, M. A.; Wise, R. A.: Circling from intracranial morphine applied to the ventral tegmental area in rats. Brain Res. Bull. 11:295–298; 1983.
6. Jenck, F.; Bozarth, M. A.; Wise, R. A.: Contraversive circling induced by ventral tegmental microinjections of moderate doses of morphine and [D-Pen^2, D-Pen^5]enkephalin. Brain Res. 450:382–386; 1988.
7. Koshikawa, N.; Kitamura, M.; Kobayashi, M.; Cools, A. R.: Contralateral turning elicited by unilateral stimulation of dopamine D_2 and D_3 receptors in the nucleus accumbens of rats is due to stimulation of these receptors in the shell, but not the core, of this nucleus. Psychopharmacology (Berlin) 126:185–190; 1996.
8. Messier, C.; Mrabet, O.; Destrade, C.: Locomotor bias produced by intra-accumbens injection of dopamine agonists and antagonists. Pharmacol. Biochem. Behav. 41:177–182; 1991.
9. Paxinos, G.; Watson, C.: The rat brain in stereotaxic coordinates, 2nd ed. New York: Academic Press; 1986.
10. Smith, I. D.; Sutton, M. A.; Beninger, J. R.: Rotational bias in intact rats following intrastriatal injections of dopaminergic drugs. Pharmacol. Biochem. Behav. 58:431–441; 1997.
11. Smith, I. D.; Todd, M. J.; Beninger, R. J.: Glutamate receptor agonist injections into the dorsal striatum cause contralateral turning in the rat: Involvement of kainate and AMPA receptors. Eur. J. Pharmacol. 301:7–17; 1996.
12. Thanos, P. K.; Jhamandas, K.; Beninger, R. J.: *N*-Methyl-D-aspartate unilaterally injected into the dorsal striatum of rats produces contralateral circling: Antagonism by 2-amino-7-phosphonoheptanoic acid and *cis*-flupenthixol. Brain Res. 589:55–61; 1992.
13. Ungerstedt, U.: Striatal dopamine release after amphetamine or nerve degeneration revealed by rotational behaviour. Acta Physiol. Scand. Suppl. 367:49–68; 1971.
14. Voorn, P.; Jorritsma-Byham, B.; Van Dijk, C.; Buijs, R. M.: The dopaminergic innervation of the ventral striatum in the rat: A light- and electron-microscopical study with antibodies against dopamine. J. Comp. Neurol. 251:84–99; 1986.