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BRIEF COMMUNICATION

A Novel Procedure for Dissociating the Anatomical Bases of the Behavioral Effects of Amphetamine

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TOWELL, A , P WILLNER AND R MUSCAT *A novel procedure for dissociating the anatomical bases of the behavioral effects of amphetamine* PHARMACOL BIOCHEM BEHAV 28(3) 423–426, 1987 —A novel operative procedure is described in which the same cannula may be used to administer drugs either to the caudate nucleus (CN) or to the nucleus accumbens (NAcc) of the rat Microinjections of amphetamine (10 or 20 μg) into the CN produced a reliable and robust stereotyped response, when administered to the NAcc of the same animal the higher dose increased locomotor activity The stereotypy following peripheral administration of amphetamine (5 mg/kg) was antagonised by infusions of haloperidol (30 μg) into the CN but not into the NAcc Conversely, the locomotor activity following a lower dose of amphetamine (1 mg/kg) was antagonised by infusions of haloperidol (5 μg) into the NAcc but not into the CN The results confirm earlier reports that different anatomical structures mediate the behavioral effects of low and high doses of amphetamine

Amphetamine Haloperidol Locomotor activity Stereotyped behavior Dopamine
Caudate nucleus Nucleus accumbens Rat

THE behavioral profile of amphetamine is well documented· lower doses produce locomotor activity whilst higher doses produce a characteristic sequence of stereotyped behaviors [15,16]. Because the behavioral response to amphetamine has been widely used as an animal model of schizophrenia and mania [7], considerable effort has been made to understand the neural mechanisms that mediate amphetamine-induced behaviors. Pharmacological and biochemical studies have shown that these effects depend primarily upon brain dopamine (DA), and a number of studies have attempted to localise specific behaviors to specific brain loci Two strategies that have been used to elucidate these effects are the use of neurotoxin-induced lesions and the administration of amphetamine and other drugs into discrete brain areas. These studies indicate that amphetamine-induced locomotor activity is mediated primarily by DA neurons in the nucleus accumbens (NAcc) whereas amphetamine-induced stereotypy is mediated primarily by DA neurons in the caudate nucleus (CN) [1–6, 8–10, 12–14] However, all of this evidence is derived from studies in which the behavioral response to amphetamine is examined after manipulation of one or other of the two major forebrain DA systems This has the considerable disadvantage of increasing the variability in behavioral response between animals. We now report on a cannulation technique that allows the manipulation of both DA systems in the same animal. In the first experiment, the behavioral response to amphetamine was examined following administration to either the CN or the NAcc, while in the second experiment the effects of haloperidol administration to these two structures were examined following peripheral amphetamine challenges

METHOD

Subjects

A group of eight Lister Hooded rats (Olac, Bicester, Oxon) weighing approximately 300 g at the time of surgery, were housed individually under conditions of controlled temperature and humidity, on a 12 hr light-dark cycle (09.00 hr to 21.00 hr light) Animals were fed with standard laboratory diet (Dixon, Ware, Herts) and water was also freely available at all times

Procedure

Between 14.00 hr and 16.00 hr each day animals were removed from their home cage and placed individually in a locomotor chamber (75×75×18 cm). Eight infrared beams at a height of 5 cm divided the chamber into a grid of 15 cm squares, and beam crossings were recorded using a BBC microcomputer Test sessions were 10 min in duration and every 30 sec an assessment of stereotypy was made according to the scale of Creese and Iversen (1974). Animals were habituated to the chamber for a total of 2 hr, consisting of six pre-surgical and six post-surgical test sessions

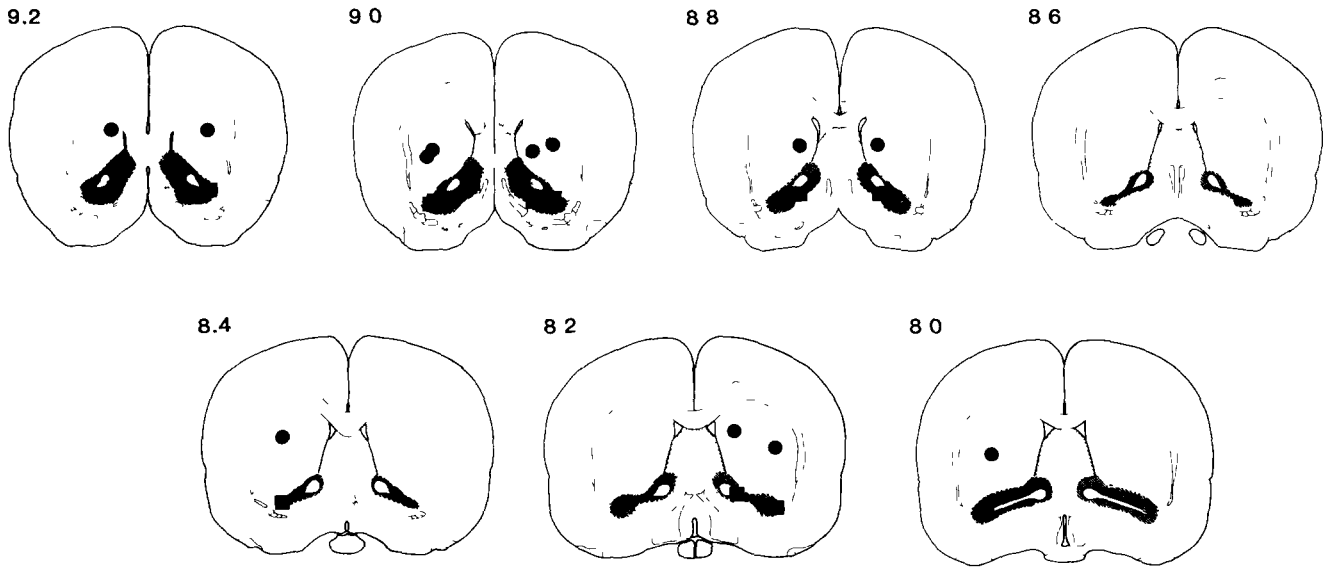

FIG 1 Serial sections through the rat brain, derived from the atlas of Pellegrino and Cushman [11] The NAcc is shaded, the CN is the structure lying dorsal to the NAcc and ventral to the lateral ventricles The positions of cannula tips in the CN are shown by circles and injection sites in the NAcc by squares

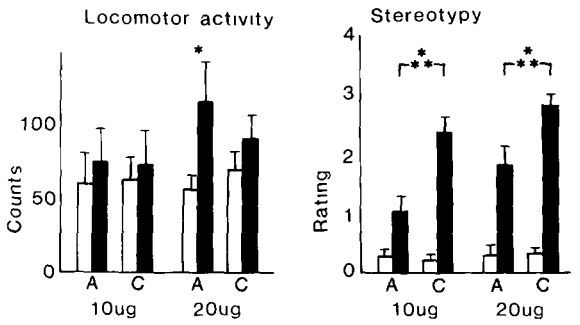

FIG 2 Locomotor counts (left) and stereotypy ratings (right) following administration of vehicle (white) or amphetamine (black) to nucleus accumbens (A) or caudate nucleus (C) Values are means (+standard error) Stars represent significant drug or site of injection effects one star, $p<0$ 05, two stars, $p<0$ 01, three stars, $p<0$ 001

Cannulae aimed at the CN were implanted bilaterally under halothane anaesthesia [17]. The co-ordinates, chosen according to the atlas of Pellegrino and Cushman [11] were anterior 8.8 mm, medial 2.5 mm and ventral 2 5 mm The cannulae were of 26-gauge stainless steel; injections through them were made using a Hamilton microsyringe with a 33-gauge needle A needle of the same (15 mm) length as the guide cannula was used for CN injections and a needle protruding 3 mm beyond the cannula tip was used for NAcc injections. Two animals died during the course of experiment 2; at the end of the experiments, injection sites were verified histologically for the other six animals All dorsal sites were located centrally within the CN (Fig. 1). Ventral sites were all within the boundaries of the NAcc as defined by Pellegrino and Cushman [11], though some workers would consider the four most posterior sites (3 animals) to be in the substantia innominata. Detailed inspection of the data did not indicate any differential drug effects related to anterior-posterior cannula placement. Spread of drugs from the injection sites was not investigated, but clear dissociations were obtained between effects at dorsal and ventral sites (see below)

Drug trials began ten days after surgery d-Amphetamine sulphate (Smith Kline and French) was dissolved in physiological saline and haloperidol (Janssen) was dissolved in a minute quantity of glacial acetic acid and made up to volume with pH 7 sodium phosphate buffer (final pH=6) In experiment 1, amphetamine was administered IC directly before testing, first at 10 μg in 0.44 μl (two closely spaced 0 22 μl pulses), and subsequently at 20 μg in 0.22 μl (one pulse); in both phases, amphetamine and vehicle were administered at both injection sites In experiment 2 amphetamine was administered IP at 1 0 or 5.0 mg/kg, in a volume of 1 ml/kg 30 minutes prior to testing. Haloperidol injections were given IC, at both injection sites, directly before peripheral amphetamine injections; a dose of 5 μg haloperidol was used with the lower dose of amphetamine, and 30 μg haloperidol with the higher dose of amphetamine, both delivered as a single pulse in a volume of 0 22 μl. In each experiment treatments were counterbalanced across animals, and a minimum of two drug-free days were allowed between successive treatments

Analysis

Results were analysed by analysis of variance supplemented by tests of simple main effects. For initial analysis of stereotyped behavior, the twenty stereotypy ratings obtained in each session were summed to give a mean stereotypy score; we have previously noted that stereotypy scores calculated in this way are normally distributed, and therefore suitable for analysis by parametric tests [18] In order to examine the distribution of stereotypy ratings, the 6-point scale was divided into three ranges. 0 (asleep or stationary); 1–2 (active, including locomotion, but with only occasional bursts of stereotyped behavior); 3–6 (continuous

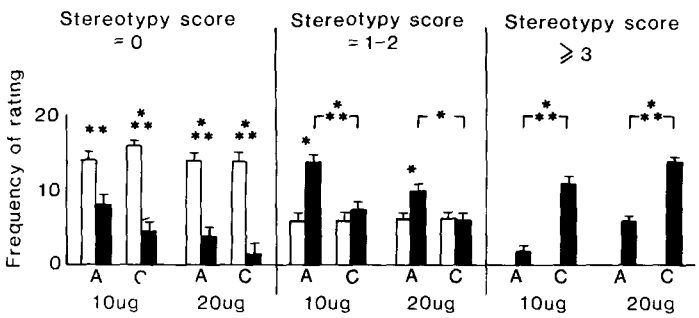

FIG 3 Distribution of sterotypy ratings after vehicle (white) or amphetamine (black) administration to the nucleus accumbens (A) or caudate nucleus (C) No scores in the highest range (right panel) were ever recorded after vehicle treatment (hence, no white bars) Values are means (+standard error) Stars represent significant drug or site of injection effects one star, $p<0$ 05; two stars, $p<0$ 01, three stars, $p<0$ 001

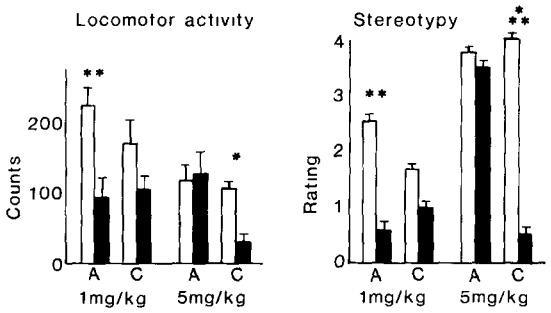

FIG 4 Locomotor counts (left) and stereotypy ratings (right) following systemic injection of amphetamine at 1 or 5 mg/kg, combined with the administration of vehicle (white) or halperidol (black) to nucleus accumbens (A) or caudate nucleus (C) Values are means (+standard error) Stars represent significant effects of drug or site of injection. one star, $p<0$ 05, two stars, $p<0$ 01, three stars, $p<0$ 001

stereotypy) The frequencies of observations within each range were then subjected to a further four-way analysis of variance with the factors drug/vehicle, doses (2), injection sites (2), score ranges (3). In a final set of analyses, the effects of peripheral amphetamine (experiment 2) were assessed in relation to vehicle scores recorded in experiment 1

RESULTS

Experiment 1

The effects of amphetamine were dose- and site-dependent (Fig. 2). Locomotor activity was increased only by administration of the higher dose (20 μg) to the NAcc, $F(1,14)=10$ 4, $p<0.01$. Stereotypy ratings were significantly increased in all four amphetamine sessions, $F(1,7)=401.0$, $p<0.001$, the effects being greater at the higher dose, $F(1,14)=5$ 5, $p<0.05$. However, in contrast to the effects on locomotor activity, stereotypy scores were increased more by administration to the CN than to the NAcc, $F(1,14)=113.0$, $p<0$ 001. Examination of the distribution of stereotypy ratings revealed a further qualitative difference between CN and NAcc (Fig 3): the predominant response to NAcc injections was a score of 1 or 2 (representing active behaviors but only occasional bursts of stereotypy), scores of 3 or more (continuous stereotypy) were very infrequent, particularly at the lower dose, $F(1,28)=15$ 9, $p<0$ 001 After CN injections, however, the predominant response was in the higher range, $F(1,28)=21$ 9, $p<0$ 001, and indeed, ratings in the intermediate range were no more frequent after amphetamine than after vehicle injections, $F(1,21)=0.01$, N S

Experiment 2

Relative to vehicle scores recorded in Experiment 1, systemic administration of amphetamine at 1 mg/kg caused a substantial increase in locomotor activity, $F(1,33)=47$ 3, $p<0.001$, and a modest increase in stereotypy scores, $F(1,33)=210.9$, $p<0$ 001, resulting largely from ratings in the intermediate (1–2) range. Five mg/kg, on the other hand, caused only a small increase in locomotor activity, $F(1,33)=6$ 6, $p<0.05$, but elicited intense stereotyped behavior, $F(1,33)=844.0$, $p<0.001$. Administration of haloperidol (5 μg) to the NAcc significantly reduced the effects of 1 mg/kg amphetamine on locomotor activity, $F(1,10)=18$ 4, $p<0$ 001, and stereotypy ratings, $F(1,10)=47$ 1, $p<0.001$ Conversely, the effects of 5 mg/kg amphetamine on locomotor activity, $F(1,12)=5.9$, $p<0$ 05, and stereotypy, $F(1,12)=176.3$, $p<0$ 001, were suppressed by haloperidol (30 μg) administered to the CN. However, administration of haloperidol to the CN did not significantly modify the effects of 1 mg/kg amphetamine, neither did administration of haloperidol to the NAcc significantly modify the effects of 5 mg/kg amphetamine (Fig. 4)

DISCUSSION

To our knowledge, this is the first study in which functional comparisons have been made by using the same cannula to administer drugs to two different dopamine terminal regions within the same animal.

One earlier study reported a blockade of amphetamine-induced stereotypy by haloperidol administration to the nucleus accumbens which was not observed in the present study There is no obvious reason for this discrepancy. The injection sites were slightly more posterior in the present study, but anterior-posterior distribution of the sites did not appear to influence the results, and in any case, the majority of sites were similarly located in the two studies. In the earlier study, haloperidol injections were made in a substantially larger volume (1 μl), which may have resulted in diffusion of the drug to the caudate nucleus [12]. This possibility is effectively excluded in the present data by the double dissociation between injection sites and drug effects. Whatever the explanation of the discrepancy between these two studies, we note that the present data are consistent with the effects of 6-OHDA lesions; amphetamine stereotypy is blocked by 6-OHDA-induced DA depletion of the CN, but not by 6-OHDA lesions of the NAcc [3, 6, 8] Furthermore, DA administration to the NAcc was found to elicit stereotyped behavior only at very high doses, which probably diffuse into the CN [5, 9, 12].

The data concur with earlier studies showing differential behavioral effects of stimulant and neuroleptic drugs applied to the CN and NAcc [1–4, 6, 10–12] Administration of amphetamine to the NAcc elicited locomotor activity and other active behaviors (sniffing, rearing, etc.), but very little intense stereotyped behavior, the administration of haloperidol to the NAcc suppressed the elicitation of these effects by

a low systemic dose of amphetamine. Conversely, administration of amphetamine to the CN caused intense stereotyped behavior, and haloperidol administered to the CN blocked the elicitation of stereotypy by a high systemic dose of amphetamine

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