Microinjections of d-amphetamine into the nucleus accumbens and caudate-putamen differentially affect stereotypy and locomotion in the rat

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For 3 days, rats received daily bilateral microinjections of d-amphetamine or saline into either the nucleus accumbens or the caudate putamen. On Days 1 and 3, each animal was tested for stereotypy and locomotion (number of grid crossings). Microinjections of d-amphetamine into the nucleus accumbens but not the caudate putamen produced an increase in locomotion but had no effect on stereotypy, whereas d-amphetamine microinjections into the caudate-putamen but not the nucleus accumbens produced an increase in stereotypy but had no effect on locomotion. The results are discussed in terms of the role of limbic and striatal dopamine systems in mediating these behaviors.

Systemic administration of d-amphetamine in the rat produces a dose-dependent sequence of behavioral effects. With increasing doses, the behavior changes from predominantly forward locomotion to a complex response pattern consisting of a prephase during which locomotor activity gradually decreases to zero, a stereotypy phase during which sniffing, biting, licking, and head movements predominate, and an after-phase during which well-coordinated forward locomotion predominates (Randrup, Munkvad, & Usden, 1963; Schiorring, 1979).

Because the behavioral response to d-amphetamine is one of the most widely used animal models of schizophrenia (Groves & Rebec, 1976; Kokkinidis & Anisman, 1980; Meltzer & Stahl, 1976), considerable effort has been devoted to understanding the neural mechanisms that mediate d-amphetamine-induced behaviors. One fundamental question is whether d-amphetamine's varying behavioral effects are produced by the drug's action on different brain areas. A number of researchers have suggested that d-amphetamine-induced locomotor activity is mediated primarily by dopamine neurons in the nucleus accumbens whereas amphetamine-induced stereotypy is mediated by dopamine neurons in the caudate-putamen (e.g., Jackson, Anden, & Dahlstrom, 1975; Kelly Saviour, & Iversen, 1975; Pijnenburg, Honig, & Van Rossum, 1975). Others have suggested that the wide variety of effects actually reflects different as-

pects of the same behavioral repertoire—the implication being that the different behaviors are not necessarily mediated by different dopamine systems (see Kokkinidis & Anisman, 1980, 1983, and Rebec & Bashore, 1982, for reviews).

There is evidence to support both viewpoints. To date, two primary strategies have been used to investigate the effects of d-amphetamine on various neural systems. The first involves examining the effects of systemic d-amphetamine injections in animals with lesions to the striatum (e.g., Costall, Naylor, & Olley, 1972). The second strategy involves recording neuronal activity in these areas following systemic d-amphetamine administration (e.g., Bashore, Rebec, & Groves, 1978). One relatively straightforward technique, which to our knowledge has not been used is to evaluate the effects of d-amphetamine, is to monitor the effects of microinjections of the drug directly into the nucleus accumbens (NA) and the caudate-putamen (CPU). The purpose of the present study was to evaluate the effects of microinjections of d-amphetamine into either the NA or CPU on locomotion and stereotypy in the rat. A second purpose of the study was to determine the effects of multiple microinjections on these behaviors.

METHOD

Subjects

The subjects were 64 experimentally naive Sprague-Dawley derived rats (Ancare, Manhasset, NY), weighing between 230 and 300 g at the time of surgery. The animals were individually housed in a colony room with a 12-h alternating light-dark cycle. Food and water were available continuously.

Apparatus

The apparatus used to monitor stereotypy and locomotor activity consisted of a large wooden box (1.2 × .65 × .5 m) with

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a Plexiglas window in the front panel door. The chamber was insulated with acoustical tile, diffusely illuminated by four overhead 28-V miniature light bulbs, and ventilated by a blower fan. Three floor-to-ceiling partitions divided the chamber into four separate compartments, each of which contained a clear plastic cage (45 × 24 × 16 cm). The bottom of each of these cages was marked in a grid such that each square was 6 × 6 cm.

Surgery

Each animal was anesthetized via an intraperitoneal injection of sodium pentobarbital (Nembutal, 50 mg/kg). A local anesthetic (Lidocaine) and atropine sulfate were administered as necessary.

The animals were placed in a Kopf (Model 900) stereotaxic instrument such that the incisor bar was 5 mm above the ear bars. For half the animals, bilateral guide cannulae were aimed at the nucleus accumbens (Pellegrino & Cushman, 1967: 3.4 mm anterior to bregma, ±1.7 mm from the midline, and 6.5 mm ventral to dura); in the remaining animals, the cannulae were aimed at the caudate putamen (1.6 mm anterior to bregma, ±3.5 mm from the midline, and 3.8 mm ventral to dura). The implants were constructed from 22-ga stainless steel hypodermic tubing embedded in a threaded nylon base (see Staton & Solomon, 1979). All guide cannulae were aimed 1 mm dorsal to the proposed injection site.

Microinjections

Half the animals in each surgical condition received a single daily bilateral microinjection of either 10 μ g (0.5 μ l, 20 μ g/liter) of d-amphetamine sulfate (Sigma Chemicals) or an equivalent volume of saline for 3 consecutive days. Microinjections were delivered by replacing the dummy cannula with a 28-ga inner cannula, which was lowered to the injection site. The inner cannula was fed by a Kopf Model 1208 microinjection unit equipped with a Hamilton 10- μ l syringe. All injections consisted of 0.5 μ l of fluid injected over a 1-min period. Following the injection, the inner cannula was left in place for 1 min to allow for diffusion.

Stereotypy and Locomotion

The animals were given a minimum of 2 weeks to recover from surgery. Testing for stereotypy and locomotion took place over a 3-day period. On Days 1 and 3, each animal was given a microinjection of either saline (SAL) or d-amphetamine (AMP) in either the NA or CPU and tested for locomotion and stereotypy. The animals were also injected on Day 2, but no testing took place. Thus, the final design consisted of four groups ($n = 16$) with two microinjection sites crossed with two drug levels.

Thirty minutes prior to injection, the animals were placed individually in the plastic observation cages. During this period, two 1-min baseline locomotor readings were taken for each animal, one at 15 min and one at 30 min. This was accomplished by counting the number of hindfoot grid crossings over the 1-min period.

At the end of the 30 min, the animals were microinjected and immediately returned to the observation cages. Then, once every 4 min for the next 32 min, each animal was observed for a 1-min period. Locomotor activity (number of grid crossings) was monitored for the first 4-min period and stereotypy was monitored during the second 4-min period, locomotor during the third period, and so on. Thus, each animal had a total of four locomotor activity readings and four stereotypy readings per day.

Numerical ratings of stereotypy were made according to the rating scale of Creese and Iversen (1974). This scale ranges from 0, indicating the animal is asleep or stationary, to 1, indicating an active animal, and then through 6, with higher numbers indicating greater degrees of stereotypy. For each 1-min period, the animals were assigned the number that corresponded to the highest degree of stereotypy shown during that period.

Histology

At the conclusion of testing, each animal was given an overdose of sodium pentobarbital and the injection site was marked by microinjecting 0.5 μ l (bilaterally) of diluted (50% with distilled water) India ink. The animals were then perfused intracardially

with 0.9% saline followed by 10% Formalin solution. The brains were removed, stored in Formalin followed by sucrose Formalin, and then embedded in albumin gelatin. Frozen coronal sections were taken at 40 μ m. The tissue was mounted and stained with cresyl violet.

RESULTS

Histological

Figure 1 shows our reconstructions of the cannula-tip placements for animals in the four treatment conditions. To localize the cannula tips, the slides of the stained tissue were placed in a photographic enlarger and the image was projected onto the corresponding plates of the atlas of Pellegrino and Cushman (1967). Cannula tip placements were identified by the location (center) of the India ink injection. Each tip placement was subsequently verified by microscopic examination of the slides. Only data from animals that had both cannulae located in the designated injection site were included in the statistical analysis. Within each condition, we were unable to ascertain any relationship between cannula tip placement and behavior.

Locomotion

Locomotor scores were calculated on each day by first taking the average number of grid crossings for the two baseline readings (B) and then calculating the average number of grid crossings for the four measurements taken during the 32-min postinjection period (P). The final locomotor score was computed

Figure 1. Cannula tip placements for animals in each of the four treatment conditions. A + B = CPU-AMP, C + D = CPU-SAL, E + F = NA-SAL, G + H = NA-SAL. Note: These marks show placement of cannula tips only. It is likely that the drug spread over a wider area.

by expressing P as a percentage of B. Thus, scores over 100% indicated an increase in grid crossing following injections.

The left panel of Figure 1 shows locomotor activity for animals in each of the four treatment conditions over the 2 days of testing. As the figure shows, there was little difference between the two saline groups (SAL-CPU and SAL-NA) and the group that received d-amphetamine in the caudate-putamen (AMP-CPU). The animals that received d-amphetamine in the nucleus accumbens (AMP-NA), in contrast, showed a substantial increase in grid crossing following injection on both Day 1 and Day 3. Statistical analysis verified these observations. A mixed analysis of variance (ANOVA) in which drug and injection site served as between-groups variables and day served as a within-groups variable revealed a significant drug $\times$ injection site interaction [$F(1,60) = 9.11, p < .001$]. Individual comparisons showed that the interaction was due to an increase in locomotor activity in Group AMP-NA. Specifically, there was no difference in the change in the number of grid crossings (collapsed across days) between Groups SAL-CPU and AMP-CPU [$t(30) < 1, p > .05$]. Group AMP-NA, however, showed a significant increase in grid crossing relative to Group SAL-NA [$t(30) = 5.30, p < .001$]. There were no interactions of the day's factor with drug, injection site, or the interaction of the two ($ps > .05$), indicating no difference between groups over the 2 days of testing.

Stereotypy

Stereotypy ratings were calculated for each animal on each day by first summing the stereotypy ratings for all four periods on a particular day and then expressing this number as a percentage of the highest possible rating. Because the highest stereotypy rating for any 1-min period was 7, the sum of each set of four ratings was divided by 28.

The right panel of Figure 1 shows these stereotypy ratings for animals in each of the four treatment con-

ditions. As the figure indicates, animals in Groups SAL-NA, SAL-CPU, and AMP-NA all showed relatively low amounts of stereotypy. Animals in Group AMP-CPU, in contrast, showed relatively high amounts of stereotypy.

Statistical analysis of the data (the same ANOVA used to analyze the locomotor data) verified these observations by revealing a significant drug $\times$ injection site interaction [$F(1,60) = 17.9, p < .001$]. Individual comparisons indicated that this interaction was due to a significantly higher percentage of stereotypy in animals in Group AMP-CPU. Individual comparisons indicated that there was a significantly higher percentage of stereotypy in Group AMP-CPU than in Group SAL-CPU [$t(30) = 4.75, p < .001$]. There was, however, no difference between Groups AMP-NA and SAL-NA [$t(30) < 1, p > .05$].

Although there appears to be a slightly higher percentage of stereotypy on Day 3 than on Day 1, this difference was not reliable. This is supported by the failure to find an interaction of the day's factor with drug, injection site, or the interaction of the two ($ps > .05$).

DISCUSSION

The data reported here indicates that microinjections of 10 μ g of d-amphetamine into the nucleus accumbens but not the caudate putamen produce an increase in locomotion but have no effect on stereotypy, whereas d-amphetamine microinjections into the caudate-putamen but not the nucleus accumbens produce an increase in stereotypy but have no effect on locomotion. Our results also indicate that there was no change in these behaviors over the 3 days that the drug was microinjected.

It is possible that the differential effects of the drug in two brain areas is due to differential sensitivities. For example, functionally higher doses in the NA might produce stereotypy that would preclude increased locomotion. There are, however, several lines of evidence to suggest that this is not the case: (1) Bashore et al. (1978) reported similar changes in single-unit activity in the NA and CPU following systemic administration of a relatively low dose (2-5 mg/kg) of d-amphetamine. (2) In 10 pilot animals, we doubled the dose of d-amphetamine injected into the NA and CPU (20 μ g, 0.5 μ liter, 40 μ g/ μ liter, n per group = 5) with no change in the results from those obtained in the 10- μ g dose.

The data from the present study are consistent with a substantial literature implicating dopamine in the NA as being important in mediating locomotion and dopamine in the CPU as being important in mediating stereotypy. This view is based primarily on studies that have found that d-amphetamine-induced stereotypy is abolished in animals with lesions to either the CPU (e.g., Costall et al., 1972) or the substantia nigra (Price & Fibiger, 1974). Neither of these lesions

Figure 2. Locomotion (left panel) and stereotypy ratings (right panel) for animals in each of the four treatment conditions.

had any effect on d-amphetamine-induced locomotor activity (Creese & Iversen, 1974; Naylor & Olley, 1972). Pharmacological studies have led to similar conclusions. For example, Pijnenburg and Van Rossum (1973) found that microinjections of dopamine into the NA produced an increase in locomotion but had no effect on stereotypy, whereas an equivalent injection into the CPU produced an increase in stereotypy but had no effect on locomotion. Jackson et al. (1975) found similar effects in the NA.

Although these data are all consistent with the view that d-amphetamine may produce its varying behavioral effects by acting on different DA systems, several recent accounts have suggested that this view may be too simplistic. Rebec and Bashore (1982) reviewed a substantial body of literature and suggested that both the NA and the CPU mediate components of d-amphetamine-induced locomotion. They also suggest that there is regional variation in the mediation of different behaviors within the CPU itself. For example, Mason, Sanberg, and Fibiger (1978) reported that d-amphetamine-induced stereotypy is not reduced by dorsal CPU lesions. It is noteworthy that in the present study we were unable to detect any relationship between injection site in either the CPU or the NA and behavior. Kokkinidis and Anisman (1980) also argue that the sites of dopamine activity responsible for locomotion and stereotypy have not been identified. They suggest that locomotion and stereotypy are likely to be mediated by the interaction of several neuroregulator systems and through a complex series of feedback loops encompassing several brain areas.

To our knowledge, this present study is the first to directly compare the effects on locomotion and stereotypy of d-amphetamine injected into the nucleus accumbens and caudate-putamen. And, although these data must be considered in the context of other recent work suggesting that the control of these two behaviors is complex, the present data do suggest that direct application of d-amphetamine into these two structures produces different behaviors. It is possible that the performance of one set of behaviors versus another during systemic administration of d-amphetamine may represent a reflection of the predominant site of action of the drug at any given point in time. The data from the present experiment are consistent with this view. These data are also consistent with results showing differential effects of d-amphetamine microinjections into these two areas on other behaviors (Solomon & Staton, 1982). Nevertheless, it remains an open question as to exactly how these behaviors are mediated and what roles various anatomical and pharmacological systems play.

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