Pharmacology Biochemistry & Behavior, Vol 28, pp 393–399 © Pergamon Journals Ltd , 1987 Printed in the U S A 0091-3057/87 $3 00 + 00

Effects of Apomorphine and Amphetamine on Patterns of Locomotor and Investigatory Behavior in Rats

MARK A. GEYER,[1] PATRICK V. RUSSO,
DAVID S. SEGAL AND RONALD KUCZENSKI

Department of Psychiatry, University of California, San Diego, La Jolla, CA 92093

Received 3 November 1986

GEYER, M A , P V RUSSO, D S SEGAL AND R KUCZENSKI *Effects of apomorphine and amphetamine on patterns of locomotor and investigatory behavior in rats* PHARMACOL BIOCHEM BEHAV **28**(3) 393–399, 1987 —Rats were tested in a Behavioral Pattern Monitor after various doses of either amphetamine or apomorphine in order to characterize their behavioral profiles, including patterns and sequences of holepokes, rearings and locomotor movements To enable direct comparisons between the behavioral effects of the two stimulants, doses and times for each drug were selected with which locomotor hyperactivity was the predominant behavioral response. Although both drugs increased the total amount of locomotor activity, amphetamine induced a relatively varied behavioral profile while apomorphine induced repetitive behavior with a restricted range of responses These contrasting effects of the stimulants were interpreted as reflective of their differing modes of action with regard to central dopaminergic systems It is suggested that, in the dose range used, the release of dopamine by amphetamine is coupled to neuronal firing and therefore this release increases behavioral activity without altering the normal response repertoire of the animal Conversely, the direct agonist action of apomorphine results in a restricted and perseverative behavioral pattern because its activation of forebrain dopamine receptors is independent of the normal physiological pattern of dopaminergic neuronal firing

Rats Locomotor activity Locomotor patterns Investigatory behavior Perseveration Holeboard Amphetamine Apomorphine

CONSIDERABLE evidence indicates that the dopaminergic innervation of the striatum is critically involved in the focused stereotypies induced by relatively high doses of stimulants such as amphetamine and apomorphine [3,10]. We and others have observed [12, 15, 16] that the characteristic feature of stereotypy, the repetition of certain behavioral elements to the exclusion of others, is also evident with moderate doses of amphetamine, especially in the form of perseverative spatial patterns of locomotion We have suggested [16] that, as with the more focused stereotypies, the striatal dopamine system may also play an important role in the perseverative quality of stimulant-induced patterns of locomotion. More specifically, we have speculated that the perseverative nature of the behavioral response to these agents reflects the dissociation of dopamine receptor activation from impulse-mediated dopamine release [11]

At relatively low doses of dopamine-releasing drugs such as amphetamine, impulse flow in mesostriatal dopamine neurons remains relatively unaltered [1,20] and amphetamine-induced dopamine release is coupled to impulse flow [19]. Under these conditions, therefore, the patterning of the dopaminergic inputs to the striatum, particularly those modulated by cortical mechanisms, may be preserved, though at an enhanced level Thus, behavioral output, while activated, retains a varied, environmentally interactive pattern At moderate to high doses of amphetamine, however, there is a dissociation of dopamine receptor activation from impulse-dependent dopamine release. That is, impulse flow in mesostriatal dopamine neurons is inhibited [1,20], amphetamine-induced dopamine release is independent of impulse flow, and activation of striatal dopamine receptors is thus nonspecific and independent of information transfer via dopamine neurons. Similarly, with all doses of direct-acting dopamine agonists like apomorphine, the activation of dopamine receptors within the striatum is not coupled to mesostriatal dopamine impulse flow

To test the hypothesis that the degree of perseveration and restrictiveness in the behavioral response profiles associated with direct and indirect dopamine agonists is related to the dissociation of receptor activation from dopamine impulse flow, we compared the effects of relatively low doses of amphetamine and apomorphine. Following the administration of saline or one of several doses of apomorphine or amphetamine, rats were tested in a behavioral pattern monitor (BPM). At doses and time intervals selected to

[1]Requests for reprints should be addressed to Mark A Geyer, Ph D , Department of Psychiatry, T-004, University of California, San Diego, La Jolla, CA 92093

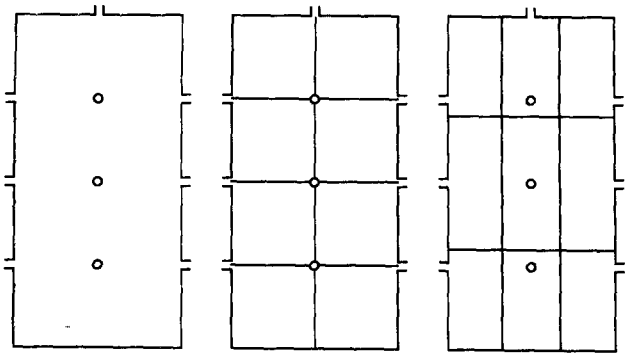

FIG 1 Diagrammatic representation of the Behavioral Pattern Monitor chamber The positions of the seven wall and three floor holes are shown in each of the three diagrams (a) Photobeams Infrared photobeams are arranged in a cartesian coordinate system on 7 6 cm centers and are sampled ten times per second (b) Sectors Sectors are 15 2 cm squares Crossovers are defined as movements between any of these Sectors (c) Regions Regions are unequal in size and are used primarily to define entries into the corners and the center

ensure that increased locomotion was the predominant response, behavior was assessed with respect to the sequencing and spatial patterning of the animals' locomotor movements, holepokes, and rearings.

METHOD

Animals

Male Sprague-Dawley rats weighing 275–300 g (Simonsen Laboratories, Gilroy, CA) were used. All animals were individually housed on a 12/12 hour light/dark cycle Each group was allowed a seven day period for acclimation to the animal room before behavioral testing.

Behavior Pattern Monitor Chambers

A more detailed description of the apparatus is available elsewhere [8]. Briefly, each of the eight BPM chambers consists of a 30.5×71 cm black Plexiglas holeboard with three floor holes and seven wall holes, as shown in Fig 1. Within the holeboard is a 4×8 X-Y array of infrared photobeams placed 2 cm above the metal floor. When sampled by the computer, these beams are used to define the X-Y position of the animal with 4 cm resolution. Each 2 5 cm hole is equipped with an infrared photobeam for detection of holepokes Rearing against the walls of the holeboard is detected by a touch-plate 15 cm above the floor. Every 100 msec, the computer samples the status of all the beams (and circuits) in each chamber If any change has occurred from the previous stored reading for the chamber, the current status of all beams is stored together with the number of 100 msec intervals since the previous reading. All the data are stored permanently

Procedures

For an experimental session, animals were brought to the laboratory one hour prior to testing Each animal was gently placed into an experimental chamber and the computer was signaled by a button push to start collecting data from that box The chambers were thoroughly cleaned between animals Test sessions were conducted during the dark phase of the animals' light/dark cycle and lasted 60 min. Subcutaneous injections of saline or one of several doses of the test drug were given 10 min prior to the introduction of the animal to the chamber The apomorphine study involved 60 rats in five groups given saline or the following doses of apomorphine HCl (in mg/kg salt). 0 1, 0 5, 1.0, 2 0 The amphetamine study involved 37 rats in four groups given saline or the following doses of amphetamine sulfate (in mg/kg free base). 0.25, 0.5, 1 0

Visual Observations

Additional animals were used for visual observations. The above procedure was followed with the exception that a 15 watt red light illuminated the BPM. Animals were rated by trained observers unaware of treatment conditions through fish-eye viewing lenses mounted in the lid of the enclosure Behavioral ratings were recorded for a 1 min period every 4 min

Data Reduction

Data reduction took place in two stages, one in which responses were counted per unit time and the other in which the sequential patterns of movements were assessed throughout the test sessions. For the first analysis, the raw data were translated into frequency and durations of events cumulated over 5-min epochs During this pass, X-Y position was calculated and used to define an animal's position in one of eight equally sized sectors (Fig. 1b) and one of nine unequally sized regions (Fig. 1c) Crossovers required whole body translocations for scoring, being defined as the number of transitions between any of the 15 cm square sectors (Fig 1b). Center duration was defined as the accumulated time spent in the center region (Fig. 1c)

The measure of corrected holepokes was calculated by dividing the total number of holepokes by crossovers in order to adjust holepokes for the amount of locomotor activity Repeated holepokes were defined as the number of consecutive holepokes into the same hole which were not separated by an intervening crossover, rearing, or a holepoke into a different hole, all other holepokes were defined as varied holepokes. The ratio of repeated to varied holepokes was also calculated, a measure which is effectively self-correcting for the level of activity. Corrected rearings were defined as for holepokes.

X-Y Plotting

For the second form of analysis, the raw data were translated into a sequence of X-Y positions, together with a time code and a response code, which were stored in a diskfile for each animal. The operator could then request a moving video display of the animal's X-Y position changes, rearings and holepokes at any rate from 20 to 1 times the real-time speed. Thus, an hour session could be condensed into as few as 3 min. The display could be stopped, resumed, or restarted at any time Typically, a string of the ten most recent responses was displayed This form of information greatly facilitated the human recognition of sequential patterns Reconstructions of the X-Y movements on paper were accomplished with a Zeta Plotter using a Fortran program which excluded rearings and holepokes and randomly introduced ±40% "noise" in the X and Y values to minimize exact retracings of the same line.

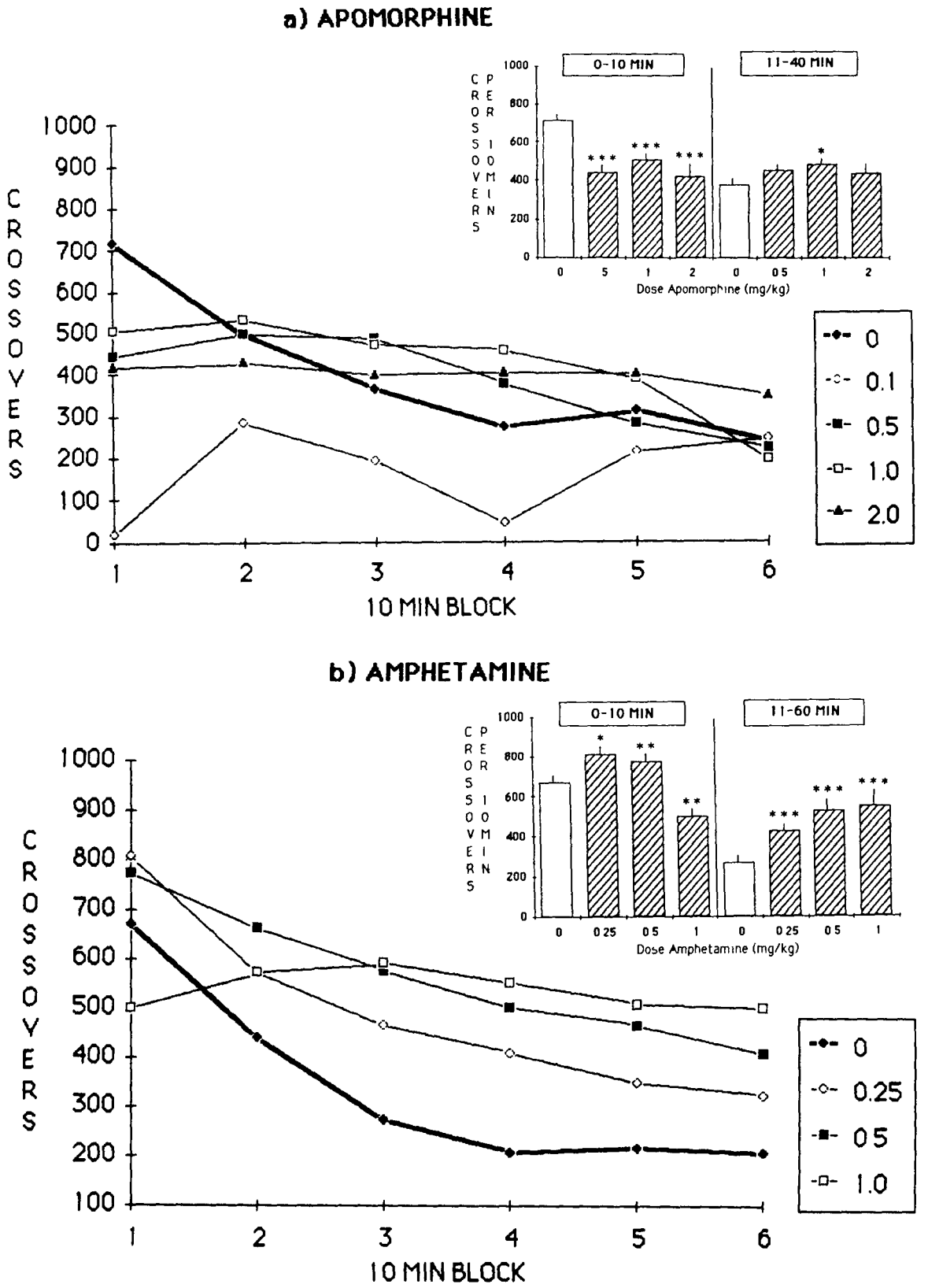

FIG 2 Time course of the effects of (a) apomorphine and (b) amphetamine on Crossovers The effects of the selected doses of the two stimulant drugs on crossovers per 10 min are shown as group (n=9–12) means at the indicated doses Inset group means±SEMs, for apomorphine at 11–40 min, and for amphetamine at 11–60 min Significance $*p<0.05$, $**p<0.01$, $***p<0.001$

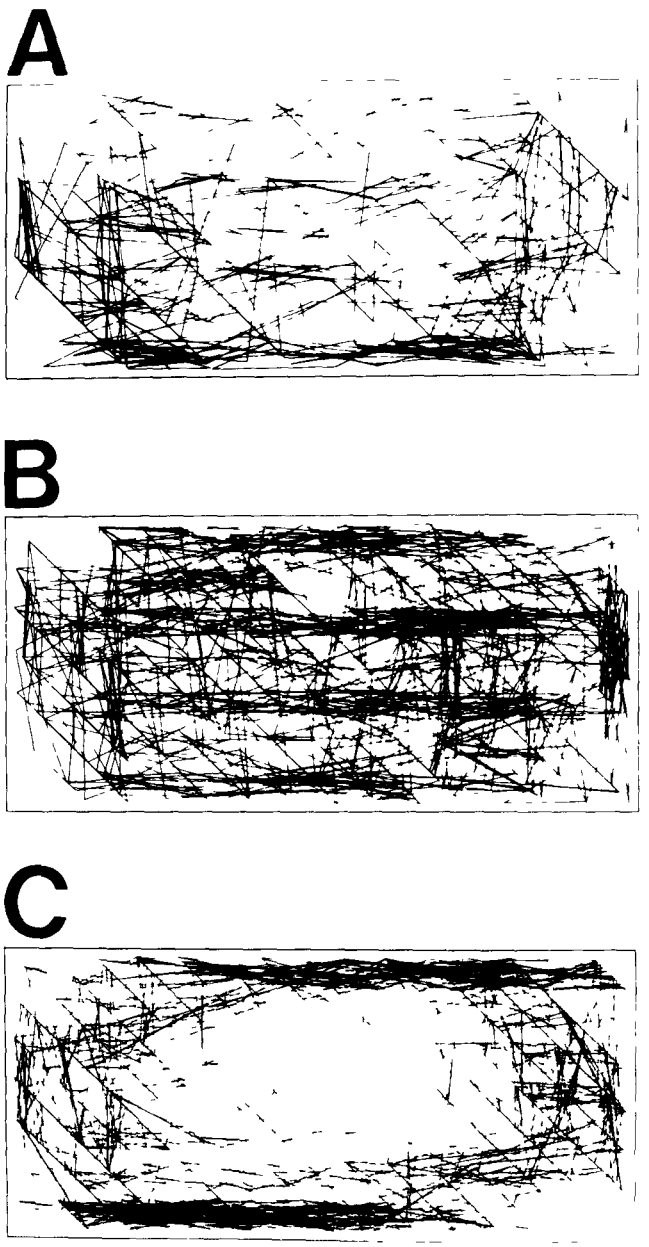

FIG 3 Spatial patterns of locomotion exhibited by stimulant-treated animals Shown here are the computer-reconstructed movement patterns exhibited by representative animals given saline (upper panel), amphetamine (middle panel), or apomorphine (lower panel) Each plot represents the activity from minutes 11 to 40 after the animal was placed in the chamber

Data Analysis

After reduction, selected variables were transmitted to the University's VAX computer for statistical analyses, using the Biomedical Computer Programs (BMDP) [4] Repeated-measures and mixed-design analyses of variance were performed for selected variables using BMDP2V. The criterion for significance was $p<0$ 05.

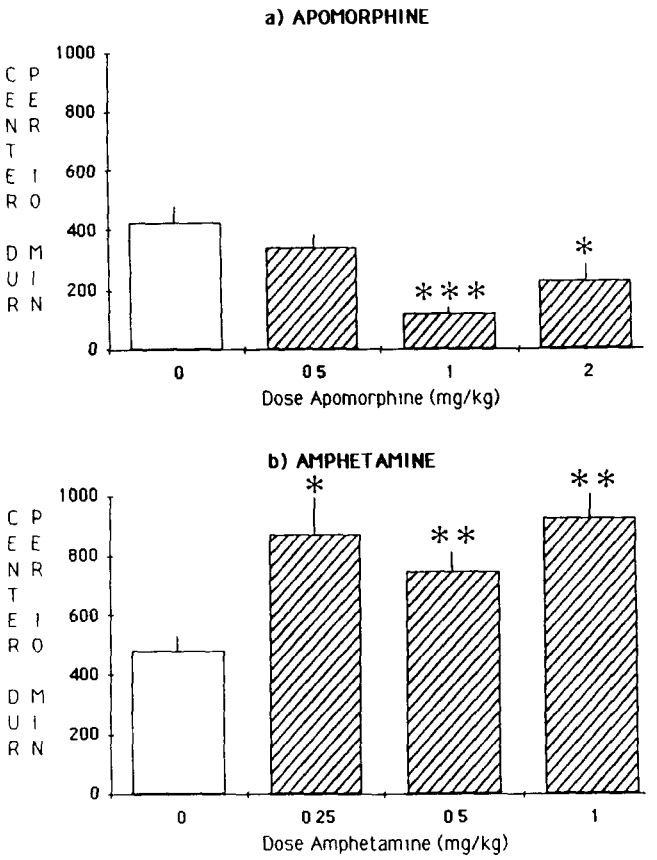

FIG 4 Effect of (a) apomorphine and (b) amphetamine on center duration per 10 min Conventions are as for Fig 2 inset Values are presented in tenths of sec

RESULTS

Locomotion

Figure 2 illustrates the time course of the effects of apomorphine (a) or amphetamine (b) on crossovers resolved into 10 min blocks. Both drugs significantly altered crossovers ,across the 60 min test period· for apomorphine, $F(20,245)=5$ 25, $p<0.0001$, for amphetamine, $F(15,160)=9$ 65, $p<0.0001$ In order to compare the effects of apomorphine versus amphetamine on investigatory behavior and patterns of locomotion, dose and time ranges were selected from Fig 2 in which increased locomotion was the predominant response. Therefore, the data have been condensed into those time blocks reflecting the hyperactive phase of each drug effect. 11–40 min for apomorphine, 11–60 min for amphetamine. Also, the 0 1 mg/kg dose of apomorphine was excluded since it consistently decreased locomotion.

Apomorphine (Fig. 2a inset) significantly increased mean crossovers above control up to about 125% in the time range 11–40 min, $F(3,40)=10$ 48, $p<0.0001$, though not dose-dependently. Conversely, apomorphine produced a significant decrease in locomotion in the first 10 min, $F(3,40)=9.89$, $p<0$ 0001. Amphetamine (Fig. 2b inset) produced a significant, $F(3,33)=25.26$, $p<0.0001$, dose-dependent increase in locomotion in the time range 11–60 min, reaching a two-fold increase over control at the 1.0

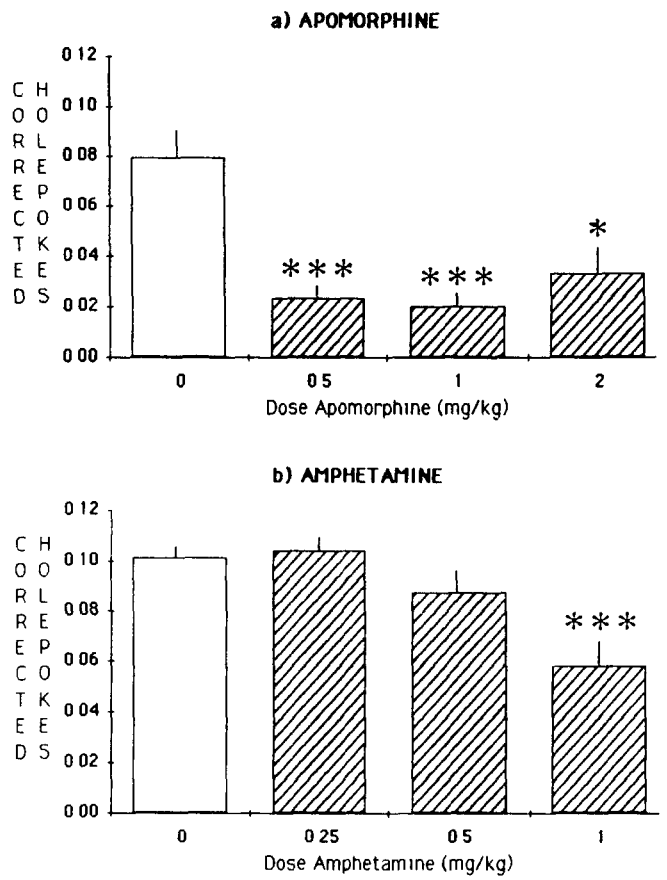

FIG 5. Effects of (a) apomorphine and (b) amphetamine on holepokes divided by crossovers (corrected holepokes) Conventions are as for Fig 2 inset

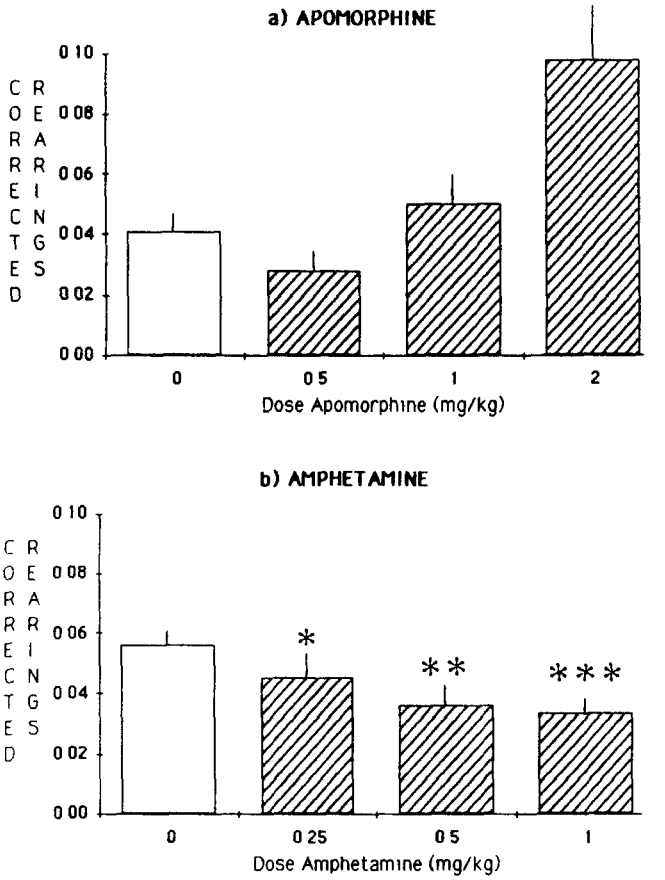

FIG 6. Effects of (a) apomorphine and (b) amphetamine on rearings divided by crossovers (corrected rearings) Conventions are as for Fig 2 inset

mg/kg dose. Depending on dose, crossovers in the first 10 min either increased or decreased.

Locomotor Pattern

Apomorphine induced perseverative or repeated spatial patterns of locomotion in most animals By visual observations of selected animals and of computer reconstructions of the animals' movements on a video terminal, the key feature of this pattern was noted to be circling in one direction around the perimeter of the chamber in a head-down, snout-contact position [2,5], with a concomitant exclusion of the center region (Fig 3, bottom panel) from about minute 5 or 10 to minute 40 of the session [12]. This exclusion of the center was reflected in a significant, but not dose-related, decrease in the center duration, $F(3,40)=5.67$, $p<0.005$, (Fig. 4a). While exhibiting this pattern of behavior, the animals rarely reared or investigated the holes. While this pattern was seen with most apomorphine-treated rats, some animals, especially with the higher doses, exhibited relatively localized movements in one part of the chamber, typically dominated by frequent and stereotyped rearing responses. On the other hand, amphetamine (Fig. 3, middle panel) in the selected dose range produced highly varied patterns of directional changes In contrast to the thigmotaxis exhibited to some extent by controls and to a greater extent by apomorphine animals (Fig. 4a), amphetamine-treated rats exhibited a more distributed occupancy of the chamber which significantly increased center duration (Fig. 4b), $F(3,33)=3\ 66$, $p<0.02$

Holepokes

As previously reported [6,9], apomorphine significantly decreased the number of holepokes corrected for level of activity (Fig. 5a), $F(3,40)=12.09$, $p<0.0001$, an effect that was independent of dose. Amphetamine also decreased the corrected holepokes (Fig. 5b), but in a dose-related manner, $F(3,33)=6.95$, $p<0.001$. Holepokes were further separated into repeated and varied holepokes. Whereas amphetamine significantly decreased the ratio of repeated to varied holepokes, apomorphine had no significant effect on this measure, amphetamine: $F(3,33)=3.70$, $p<0.05$; apomorphine· $F(3,40)=2\ 21$, $p>0.1$; data not shown

Rearings

Apomorphine produced no significant change in corrected rearings, $F(3,40)=1.15$, n.s., in part because of the large variance in the effects of this drug on rearings, whereas amphetamine elicited a dose-related decrease, $F(3,33)=3\ 10$, $p<0.05$, as shown in Fig. 6. It is important to note, however, that the apomorphine-treated animals that made a large number of rearings did so in a highly perseverative fashion (data not shown)

DISCUSSION

The effects of low doses of apomorphine and amphetamine were compared on patterns of locomotion and investigation in order to evaluate the possible role of the mesostriatal DA system in regulating the behavioral variation. While both stimulants produced significant increases in the number of crossovers, amphetamine was considerably more effective in increasing this measure of the amount of behavioral activation In addition, marked differences were observed in the spatial patterns of locomotion engendered by the two treatments (Fig. 3) With apomorphine, the animals exhibited highly repetitive, unidirectional patterns of movement which were typically restricted to the periphery of the chamber. In contrast, amphetamine-treated animals frequently changed directions, resulting in patterns which were non-repetitive and varied in appearance In addition, their activity was widely distributed throughout the chamber, including relatively long periods of time spent in the center region (Fig. 4).

The two drugs also produced differential effects on holepokes, a frequently used measure of investigatory behavior. Although the ratio of holepokes to crossovers (corrected holepokes) was significantly decreased by both drugs, amphetamine decreased the ratio of repeated to varied holepokes, whereas apomorphine failed to affect this measure. This effect of low doses of amphetamine is consistent with other reports [6,9] and contrasts with the effects of higher doses of amphetamine [14].

Hence, the multivariate assessment of behavior provided by the BPM system revealed a number of differences in the locomotor-exploratory profiles elicited by the indirectly acting dopamine agonist amphetamine relative to the direct agonist apomorphine. In general, the behavioral profile exhibited by apomorphine-treated animals was relatively restricted and perseverative in nature These animals rarely investigated the holes in the chamber and typically limited their activity to the perimeter of the chamber, circling in a unidirectional pattern. By contrast, amphetamine-treated animals exhibited behaviors which were more varied both with respect to rearings and to investigating the holes. In addition, they spent more time in the center of the chamber and made more directional changes when locomoting than did apomorphine-treated animals These results are consistent with a role for mesostriatal DA systems in the regulation of behavioral variation. Specifically, the relatively normal variation in the behavioral profile of amphetamine-treated animals may result because at low doses amphetamine-induced release of striatal dopamine appears to be dependent upon neuronal activity [19]. Therefore, the activation of forebrain dopaminergic systems is related to a normal pattern of impulse-mediated dopamine release, although at an enhanced level As the dose of amphetamine is increased neuronal activity in mesostriatal dopamine neurons is progressively and ultimately completely inhibited [1,20], leading to dopamine release uncoupled from impulse flow Paralleling this pattern of altered dopamine release, higher doses of amphetamine induce perseverative spatial patterns of locomotion and focused stereotypies [12, 15, 16] Consistent with our observations, since apomorphine exerts a direct activation of post-synaptic dopamine receptors, even low doses produce a perseverative and restricted behavioral profile That the actions of apomorphine were post-synaptic in the dose range tested is consistent with our finding that a lower dose (0.1 mg/kg) of apomorphine produced opposite effects on locomotor activity, presumably by preferentially interacting at the dopamine autoreceptor to inhibit the firing of dopaminergic neurons [17]. Similar conclusions were reached by Stahle and Ungerstedt [18] based on their observations of the qualitative differences in the behavioral effects of low versus high doses of apomorphine

One alternative explanation for these observed differences between apomorphine and amphetamine derives from the fact that only the latter stimulates the release of norepinephrine as well as dopamine [11]. While norepinephrine release is likely to contribute to the behavioral effects of amphetamine, other evidence suggests that it is not responsible for the relatively varied nature of the behavioral activation noted here. Based on the multivariate measures provided by the BPM, we have previously reported that neurotoxin-induced depletions of brain norepinephrine *increase* rather than decrease the variability of spatial patterns of locomotion engendered by amphetamine [7]

In conclusion, our results are consistent with the notion that a dissociation between postsynaptic dopamine receptor activation and presynaptic dopamine neuronal activity is implicated in the perseverative quality of the behavioral response to dopamine releasing agents like amphetamine [11] At low doses of amphetamine, enhanced dopamine output occurs in the presence of a relatively normal pattern of impulse flow in mesostriatal dopamine neurons. Higher doses of amphetamine, by disrupting this patterned output, produce a more restricted and perseverative behavioral response pattern

ACKNOWLEDGMENTS

This work was supported by grants from NIDA (DA-02925 and DA-01568) M A Geyer was supported by a Research Scientist Development Award from NIMH (MH-00188) and D S Segal by a Career Scientist Award from NIMH (MH-70183) We thank Virginia Masten for her help with these studies

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