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Multivariate Assessment of Locomotor Behavior: Pharmacological and Behavioral Analyses

MARK A. GEYER,[1] PATRICK V. RUSSO AND VIRGINIA L. MASTEN

Department of Psychiatry, University of California, San Diego

GEYER, M. A., P. V. RUSSO AND V. L. MASTEN. *Multivariate assessment of locomotor behavior: Pharmacological and behavioral analyses.* PHARMACOL BIOCHEM BEHAV 25(1)277–288, 1986.—A Behavioral Pattern Monitor (BPM) is described which is designed to assess the spatial and temporal sequences of the locomotor movements, investigatory holepokes, and rearings of rats. The system records these behavioral responses with 0.1 sec resolution in time and 1.5 inch resolution in space, and permanently stores all the resulting data. The sequences of these responses may then be displayed on a video terminal or on paper and are also available for the calculation of a variety of descriptive statistics. Studies are described in which rats were tested repeatedly without any pharmacological treatments or in single test sessions following the administration of saline or one of five stimulant drugs. A variety of descriptive measures of the temporal or spatial patterning of the animals' behavior are described and applied to the data resulting from the studies of the various stimulants. It is concluded that the combination of these measures enables distinctions to be made among these drugs which cannot be made on the basis of measures of the amount of locomotor activity.

Locomotor activity Locomotor patterns Investigatory behavior Habituation Holeboard
Amphetamine Caffeine Apomorphine Nicotine Scopolamine

MEASURES of the spontaneous activity of rats have been frequently used to assess the behavioral effects of drugs or other manipulations, either as strict measures of locomotor activity or as measures of context-dependent constructs such as emotionality or exploration. Most such measures allow one to conclude only that a given manipulation increases, decreases, or produces no change in the amount of measured activity. They provide little or no information regarding qualitative changes in the animals' behavior. The use of visual observations to supplement or replace automated measures provides qualitative information, but such measures are time-consuming and often criticized as being subjective and difficult to quantitate. The present report describes an automated measurement system designed to assess both the quantity and several aspects of the quality of the behavioral activity of rats. This Behavioral Pattern Monitor (BPM) provides simultaneous monitoring of several different responses as they occur in sequence and time.

Both experimental data and critical reviews of activity meaures emphasize the importance of taking multiple measures of activity concurrently. Different measurement techniques purported to measure the same behavior, horizontal locomotion for example, may yield different results depending on the particular sensitivities of the recording device. For example, Ljungberg [15] combined two standard activity monitoring devices, an Animex activity meter and a photobeam box, for simultaneous assessment of several catecholaminergic drugs. He found that the two devices reflected behavioral changes differently and that the results from the two devices were not correlated. In addition, the interrelatedness of different behaviors, at least at the level of their expression, requires the use of concurrent measures of different behaviors in order to draw conclusions about direct effects of experimental manipulations. For example, a reduction in rearing behavior could result from either motor ataxia or a selective increase in horizontal locomotion rather than a direct effect on rearing *per se*. Such possibilities can only be distinguished when both behaviors are simultaneously measured. In order to draw conclusions about any one aspect of activity, other contributions to the measured behavior must be excluded, controlled, or monitored.

Further considerations are relevant to studies of exploration or investigatory behavior, where a major difficulty has been to distinguish between activity related to an animal's internal level of arousal and activity elicited by external stimuli. Berlyne [3,4] has developed the most explicit theoretical description of the nature of exploratory behavior and the major factors influencing it. He has argued the need for the concept of exploration in part because of the many experiments demonstrating that laboratory rats react posi-

[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.

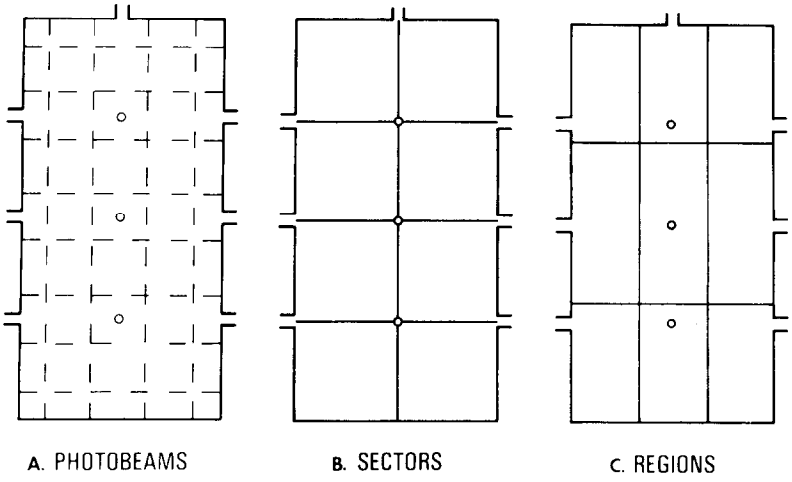

FIG. 1. Diagrammatic representation of the Behavioral Pattern Monitor chamber. The positions of the 7 wall and 3 floor holes are shown in each of the 3 diagrams. A. Photobeams. Infrared photobeams are arranged in a Cartesian coordinate system on 3-inch centers and are sampled 10 times per second. B. Sectors. Sectors are 6-inch 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.

tively to novelty, spending more time exploring unfamiliar areas of a chamber and avoiding familiar areas [3, 4, 17, 19]. According to most theorists [4,17], the amount of exploratory behavior is directly related to the novelty and complexity of stimuli in the environment, and, in reciprocity with the process of habituation, inversely proportional to the organism's prior experience with those stimuli. Considerable confusion has arisen in the literature because the same measure, locomotion in an open field for example, has been used by different investigators as an arousal measure, an emotionality measure, or an exploratory measure. To overcome this problem with standard activity measures, many researchers have begun to use holeboards to provide more specific measures of investigatory responding. A holeboard is simply an activity chamber with holes into which rats frequently poke their noses. Thus, the holes serve as specific stimuli which elicit easily measured responses. The complexity and/or novelty of these stimuli can be manipulated by placing objects in the holes. File and Wardill [7,8] have shown that holepoke measures exhibit good test-retest reliability and are valid as measures of investigation in that dishabituation of the response is elicited by the addition of a novel object to a specific hole. When locomotion measures are combined with holepoke measures, the general arousing or sedating effects of a manipulation can be discriminated from more specific effects on responsiveness to discrete stimuli. For example, while both amphetamine and apomorphine increase locomotor activity, amphetamine increases and apomorphine decreases the frequency of holepokes [12,16].

While holepokes appear to be valid measures of investigatory responding, Berlyne [3,4] suggests that a distinction should be made between specific or inspective exploration, which involves the investigation of proximal stimuli, and inquisitive or diversive exploration, which brings the animal into contact with distant stimuli. Shillito's [24] studies of exploratory behavior in both laboratory and natural environments suggest that this division is both valid and useful, since the two types of investigatory responding exhibit different stimulus requirements and rates of habituation. Thus, while the durations of individual holepokes, which can be manipulated by the introduction of novel objects to the holes [9], should provide an explicit measure of inspective exploration, such measures as the number of different holes investigated per unit time should primarily reflect the stimulus sampling aspect of diversive exploration. The latter measure, however, is partially dependent upon differences in levels of activity [21] and is therefore only interpretable when concurrent measures of locomotor activity are available.

Another approach to the study of diversive exploration is to examine the route of locomotion as the animal explores the chamber by plotting the sequence of movements [1, 6, 9, 13, 14, 23]. Schiorring [23] has used this approach to demonstrate the repetitive or stereotyped nature of the spatial patterns of locomotion exhibited by amphetamine-treated rats in an open field. Despite the recent advent of automated devices which can record such patterns, the statistical description and analysis of the resulting data has posed a difficult problem. Statistical assessments of behavioral sequences have been explored most extensively by ethologists. However, most behavioral measures cannot satisfy the criteron of stationarity required for information-statistic or Markov analyses. Indeed, habituation is often both the source of considerable non-stationarity and the object of study. The present paper examines the utility of several approaches to the statistical characterization of sequential patterns of locomotion.

The BPM apparatus described in this paper combines the features of activity and holeboard chambers and measures individual response frequencies and durations. It also records the sequences of holepokes, rearings, and locomotor movements in a Cartesian coordinate system with a resolution of 0.1 sec in time and 1.5 inches in space. Since the

record of these events is permanent, it may be used for computer reconstructions of the pattern of movements on paper or on a video display or for the calculation of descriptive statistics reflective of treatment-induced differences in these patterns. In order to assess the utility of this approach to the characterization of locomotor and investigatory behavior, the present experiments included repeated tests of untreated animals and tests of the effects of a variety of stimulant drugs. Comparisons among the effects of stimulants were undertaken to determine whether more descriptive measures of spontaneous behavior would enable distinctions to be made which are not possible with standard measures of the *amount* of activity.

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.

Apparatus

The data acquisition computer is a Z80-based system using an S-100 bus and the CP/M operating system. Data from the chambers are collected through a single parallel port. An interrupt controller and real-time clock are used to control the sampling of data. Data are written to diskfiles by a disk controller which uses direct memory access so that data may be collected concurrently with accesses to the disk. The language used, STOIC, is a public-domain derivative of FORTH, and was designed for real-time process control. STOIC, which stands for Stack-Oriented Interactive Compiler, intermixes the convenience of a high-level language with the efficiency of the machine-language subroutines required for rapid data acquisition and storage.

Behavior Pattern Monitor Chambers

Each of the eight BPM chambers consists of a 12×24 inch black Plexiglas holeboard with 3 floor holes and 7 wall holes, as shown in Fig. 1. Within the holeboard is a 4×8 X-Y array of infrared photobeams placed 3/4 inch above the metal floor. When sampled by the computer, these beams are used to define the X-Y position of the animal with 1.5 inch resolution. Each 1 inch hole is equipped with an infrared photobeam for detection of holepokes. Rearing against the walls of the holeboard is detected by a touchplate 6 inches above the floor. The walls of the chamber extend 15 inches above and 6 inches below the floor. Each of the eight chambers is enclosed in an electrically shielded and ventilated wooden box. For daytime studies, each chamber is illuminated from above by a 7.5 Watt lamp. Wide-angle lenses permit observation of the entire chamber without disturbing the animal. Each chamber is also equipped with an external pushbutton to record the time of a manipulation (e.g., injection). 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 is stored permanently in a linear stream in diskfiles, and is later reduced into the variables described below.

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 signalled by a button push to start collecting data from that box. In multi-session studies, each animal was always tested in the same chamber and at the same time of day; multiple tests were separated by 48 hours. The chambers were thoroughly cleaned between animals. In the first study, 12 untreated rats were tested repeatedly on four separate days during the light phase of the animals' light/dark cycle. In a second experiment on untreated animals, 14 rats were tested in 3 60-min sessions during their nighttime. Each experiment involving a stimulant drug included 4 or 5 groups of 10–12 animals each. 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 following doses (in mg/kg) of each drug were tested: Amphetamine, 0.25, 0.5, 1.0, and 2.0; Scopolamine, 0.125, 0.25, 0.5, and 2.0; Caffeine, 2.5, 5.0, 10.0, 15.0, and 20.0; Apomorphine, 0.1, 0.5, 1.0, and 2.0; and Nicotine, 0.0625, 0.125, 0.25, and 0.5.

Data Reduction

Data reduction took place in two stages. First, the linear stream of data readings was 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 8 equally sized sectors (Fig. 1B) and one of 9 unequally sized regions (Fig. 1C). Three types of locomotor activity measures were derived. Deltas, the total number of X-Y beam breaks, provided a measure similar to that used in standard photobeam boxes and intended to be sensitive to small, rapid movements. Crossovers were the number of sector entries, and required whole body translocations for scoring. Region entries were similar to Crossovers though based on the number of crossings between the unequally sized regions. Most typically, however, the regions are used for more descriptive measures of entries into and time spent in the center or corners.

Data Analysis

After reduction, the selected variables were transmitted over the phone line to the University's VAX computer for statistical analyses, using the Biomedical Computer Programs (BMDP) provided by UCLA [5]. Repeated-measures and mixed-design ANOVAs were performed for selected variables using BMDP2V; while multivariate correlation and regression problems used BMDP2R. All statistical comparisons reported here were derived from comparisons of the particular dose group with the corresponding control group, although some figures depict only the results from the most typical control group.

X-Y Plotting

To display the sequence of responses in simulated real-time, the raw data were first 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

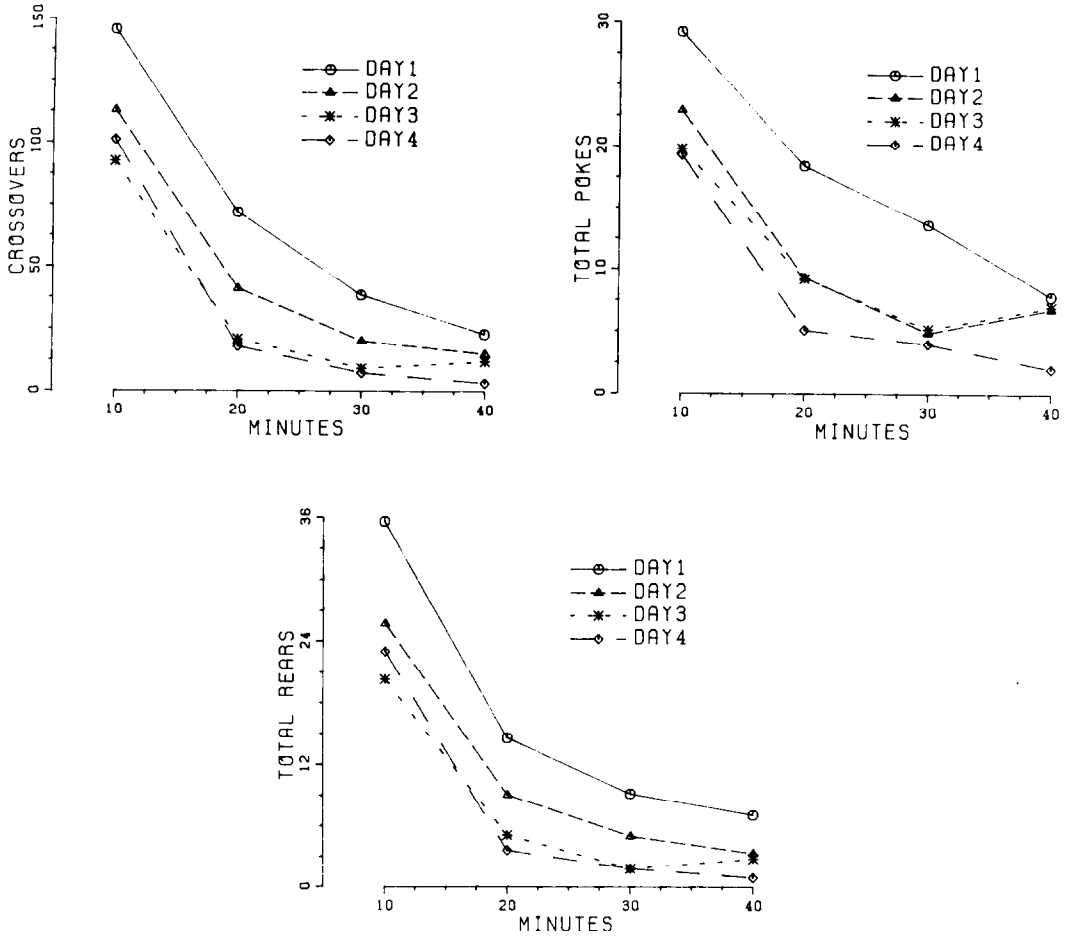

FIG. 2. Within and between session habituation curves for untreated animals. Group (N=12) means from untreated animals tested during the daytime are shown in successive 10-min blocks across 4 test sessions for Crossovers (left), Holepokes (right), and Rearings (bottom).

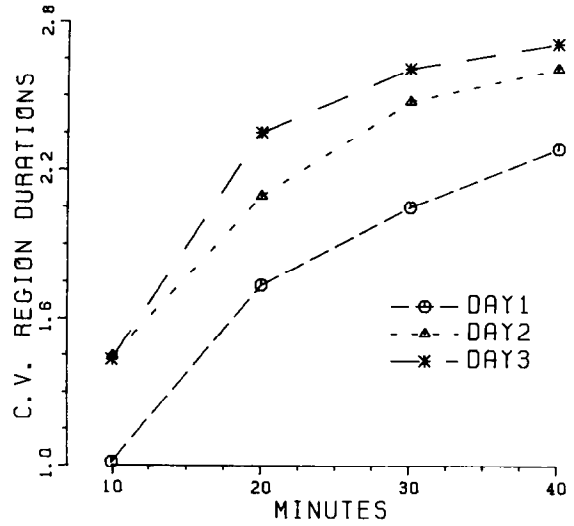

FIG. 3. Temporal CVs for untreated animals. The CV of the distribution of time spent in the 9 regions of the chamber (cf. Fig. 1C) is shown for a group of 12 untreated rats as means for 10-min blocks during each of 3 40-min test sessions. The progressive increases in the Temporal CV within each session and across days reflect the general phenomena of within- and between-session habituation.

hour session could be condensed into as few as 3 minutes. The display could be stopped, resumed, or restarted at any time. Typically, a string of the 10 most recent responses was displayed. This form of information greatly facilitated the human recognition of sequential patterns, but did not provide an output suitable for communication or publication.

To produce a hard-copy version of the X-Y movements, the X-Y data from the diskfile were converted to appropriate numbers for the University's Zeta Plotter and transmitted over the phone. Rearings and holepokes were excluded. A Fortran program then generated a Zeta plot file which controlled the plotter. To minimize exact retracings of the same line, a uniform random number generator was used to introduce ±20% "noise" in the X and Y values.

Spatial CV Measure

To statistically describe and evaluate the sequences of position changes, the data were reduced further into transitions between any of 5 areas: the 2 ends, the center, and the 2 long walls. These areas were selected to match the verbal descriptions we have found most useful in communicating an animal's preferred paths. Transitions between these areas were displayed in a 5×5 matrix with 16 permissible cells. Relative transition frequencies were then calculated as per-

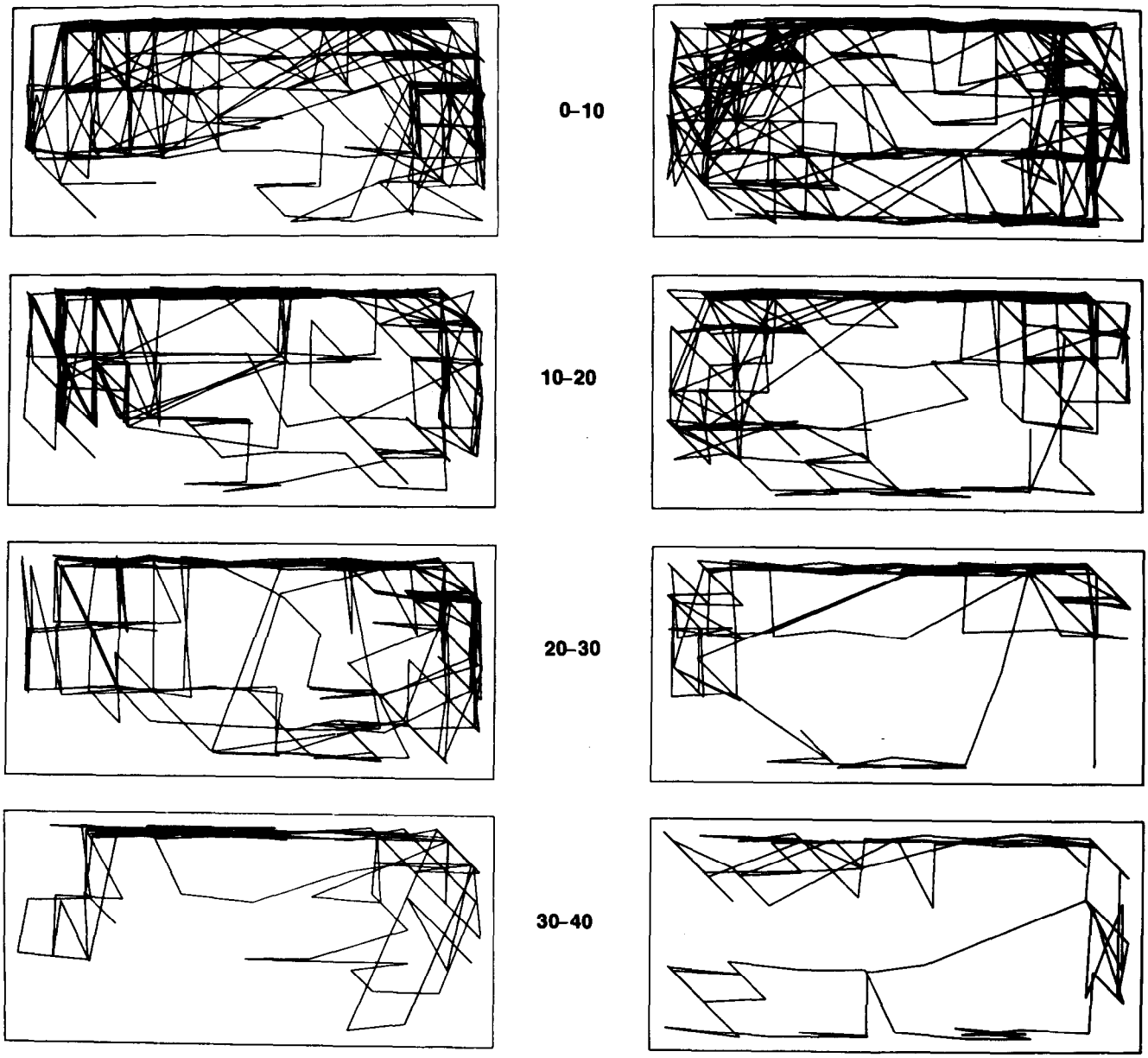

FIG. 4. Locomotor patterns in untreated rats. The computer reconstructions of the spatial patterns of movements made by two untreated rats are shown in successive 10-min segments. Within each animal, the preferred paths are consistent from one part of the session to another.

cent of total and the Spatial Coefficient of Variation (CV) was derived from this set of 16 numbers. To the extent that an animal preferentially repeated certain transitions, the Spatial CV increases. A more random distribution of these spatial transitions produces a low Spatial CV. It should be noted that this approach does not assume stationarity, as would either a Markov or an information theoretic analysis. To determine whether an animal's spatial transitions were consistent from session to session, Pearson's correlation coefficients can be calculated on the 16 pairs of values from the transition matrices.

Fractal Dimension D

This index quantifies the relative smoothness or roughness of the rat locomotor path by assessing how the *length* of the path varies across a range of measurement trials. The calculation is based on the coastline method of Richardson as described by Mandelbrot [18] which demonstrated that there was no absolute length for a given coastline, but rather an arbitrary family of possible lengths, each length being a function of the size or scale of the "ruler" used to make the length measurement. However, there is an orderly relationship between the measurement of the length and the process of measuring it, represented by the value D, the fractal dimension. In general, there is a unique value of D for each different coastline, which is attributable to its specific meandering shape, and independent of its length or the way any single measurement is made. For the present purposes, the entire rat locomotor path was treated like a coastline whose

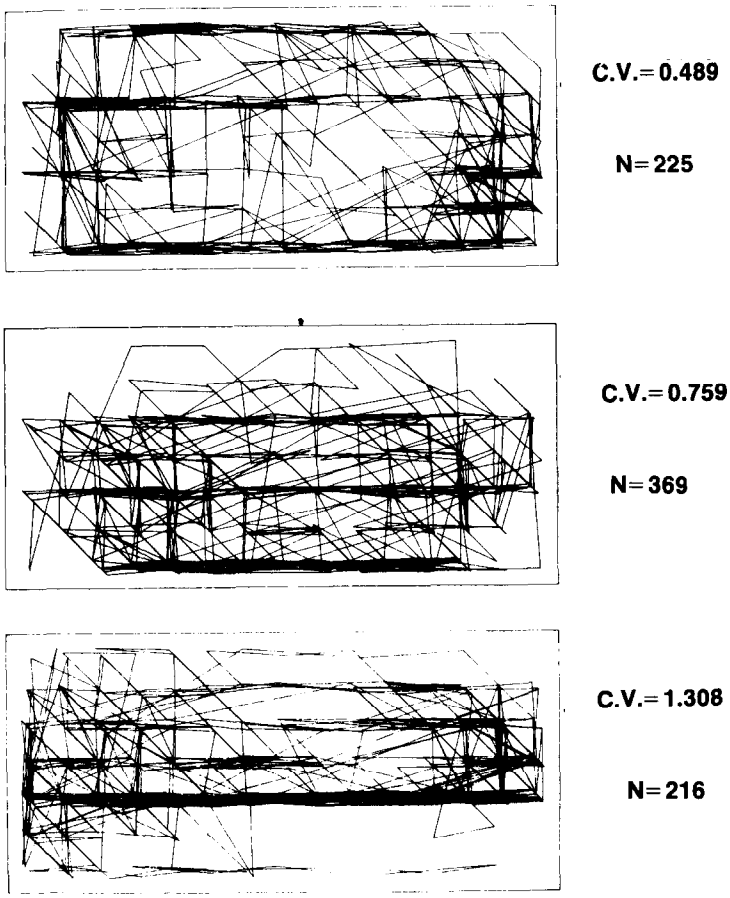

FIG. 5. Spatial CVs and movement patterns in untreated animals. The movement patterns throughout 40-min test sessions for 3 untreated animals are shown together with the calculated Spatial CV and the number of transitions from each animal.

length was then recalculated for each of a series of "rulers" of successively increasing size. A replot was then constructed of the log of these lengths versus the log of each ruler, and D was defined as 1 minus the slope of this replot.

The name fractal dimension means fractional dimension, that is, dimensions which are fractionally between the integer Euclidean dimensions of 1 or 2. In our experience, values between 1.20 and 1.30 would indicate a relatively smooth, often orderly path, values from 1.40 to 1.50 a relatively jagged or random path, and the intermediate values a path showing features of both. Since the total range of this measure is so narrow, we have found that statistically significant differences are seen with changes of as little as 0.04 units between groups. For further details of the actual mechanics of how D is calculated see the appendix in [22].

Temporal CV Measure

To complement the analyses of spatial transitions, the distribution of time spent in each of the 9 regions (Fig. 1C) was examined. While the transition analysis ignored time, this analysis focussed on the amount of time spent in each region independent of the number of entries into each region. For each animal, the distribution of time per region was determined for each 10-min block and for the entire session. As for the transition analysis, the Temporal CV was calculated as a measure of how randomly the animal distributed his time across the various regions. A high Temporal CV indicates a substantial preference for some region(s) over others. To graphically display these distributions for a group of animals, each subject's region-durations were first rank-ordered on the basis of session totals. The data were then pooled for a group independent of which regions were preferred by individuals.

RESULTS

Effects in Untreated Animals

When untreated rats are tested repeatedly at 48 hour intervals, consistent within and between session habituation curves are obtained for the standard measures of ambulation (Crossovers), rearings, and holepokes. For example, Fig. 2 illustrates these curves for a group of 12 rats tested over four sessions during the daytime. Repeated measure ANOVAs on these variables reveal significant main effects of time-blocks and days, with no significant interactions. It is apparent from Fig. 2 that the between-session habituation

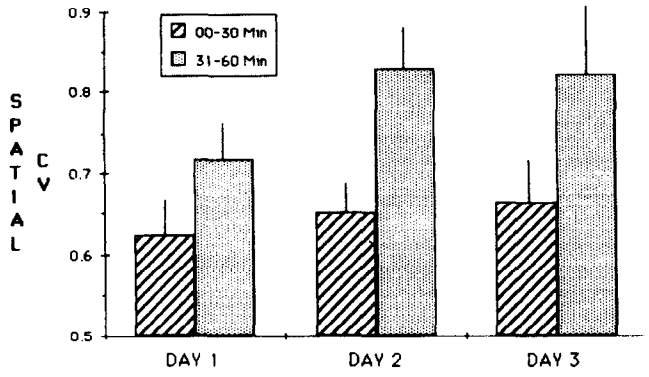

FIG. 6. Spatial CVs across days for untreated animals. The Spatial CV group (N=14) means±SEM are shown for successive halves of 3 separate hour-long test sessions. These animals were tested during their nighttime at 48-hour intervals.

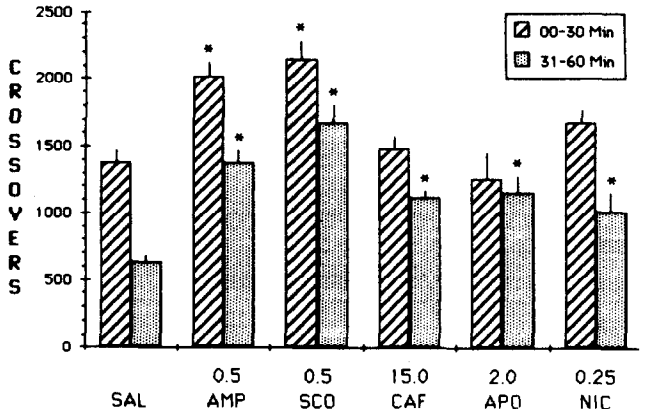

FIG. 7. Effects of stimulants on Crossovers. The effects of the selected doses of the various stimulant drugs on Crossovers are shown as group (N=10–12) means±SEM for successive halves of the hour-long test sessions. At these doses, each drug significantly increased locomotor activity during the last half of the test session. The control values shown are the median values from the separate control groups used for each stimulant study. Statistical comparisons were based on each particular control group. *=Significantly different from corresponding control, $p<0.05$.

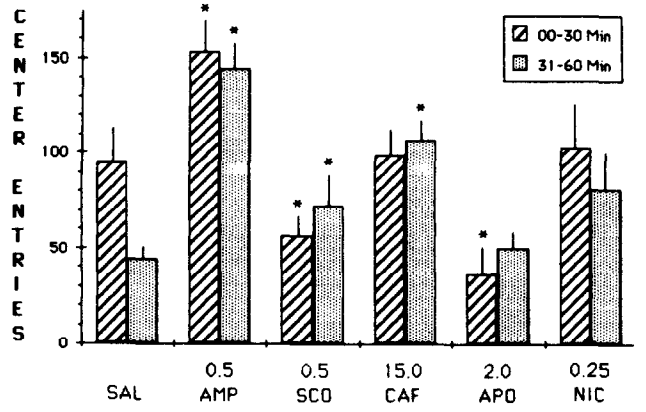

FIG. 8. Effects of stimulants on Center Entries. Group means for Center Entries are shown as in Fig. 7. The center region is illustrated in Fig. 1C. *=Significantly different from corresponding control, $p<0.05$.

decrement was most marked between the first and second session, as would be expected.

To evaluate the potential utility of the initial test sessions as "baseline" measures, between-day correlations were calculated on the session totals. To be effective in reducing variability due to individual differences, a baseline measure must reliably predict the subsequent behavior of untreated animals. Previous studies have indicated that measures of activity during the first exposure to an environment are poor predictors of subsequent individual differences relative to measures taken during the second or third exposure [10, 11, 14]. In this study as well, the second session was better correlated with sessions 3 and 4 than was session 1 with 2, 3, or 4 for virtually all measures of activity. For session 1, Pearson's r ranged from 0.32 to 0.65, while for session 2 it ranged from 0.55 to 0.75. However, session 1 was the best predictor of holepoke frequencies in subsequent sessions (r's=0.57 to 0.71).

As with the more standard measures of activity, holepokes, and rearings, the distributions of time spent in each of the nine regions (Fig. 1C) exhibited consistent within and between session trends. The Temporal CVs derived from these data appear to accurately reflect the changes in these distributions across time, as shown in Fig. 3. A repeated measure ANOVA on the Temporal CVs from the first three sessions revealed significant increases in the Temporal CV within each session, $F(3,33)=57.8$, $p<0.001$, and between sessions, $F(2,22)=12.6$, $p<0.001$, with no interaction. These increases in Temporal CV reflect the trend for each animal to spend a progressively greater proportion of his time in one or two preferred regions, generally near a corner, as his familiarity with the chamber increases. Thus, this measure reflects the general phenomenon of habituation, though it is independent of the frequencies of discrete responses.

The analyses of the spatial patterns of locomotion have revealed a remarkably consistent structure in the behavior of untreated rats. This structure was most easily identified by observation of the video displays of the sequences of holepokes, rearings, and position changes. The ability to view these sequences at fast speeds has proven to be an invaluable aid to the recognition of consistent though complex patterns of behavior. However, display rates in excess of five times normal limit one's ability to fully appreciate the durations of events such as holepokes, rearings, or pauses. As expected, there are general tendencies, which are consistent for virtually all untreated animals, to avoid the center region and prefer to stay near a corner of the chamber. Each rat, however, clearly develops his own particular spatial pattern of movements, and this pattern is predictable across time within a session and between sessions. Each animal spends most of his time in a particular corner, typically selecting the same corner on successive days. We refer to this corner as the "home" corner. From this corner, each rat makes excursions to various parts of the chamber and back, following progressively more fixed routes over time. Typically, the outward part of an excursion is less direct and more interrupted by investigatory holepoking and rearing than is the return part of the excursion. Figure 4 shows the

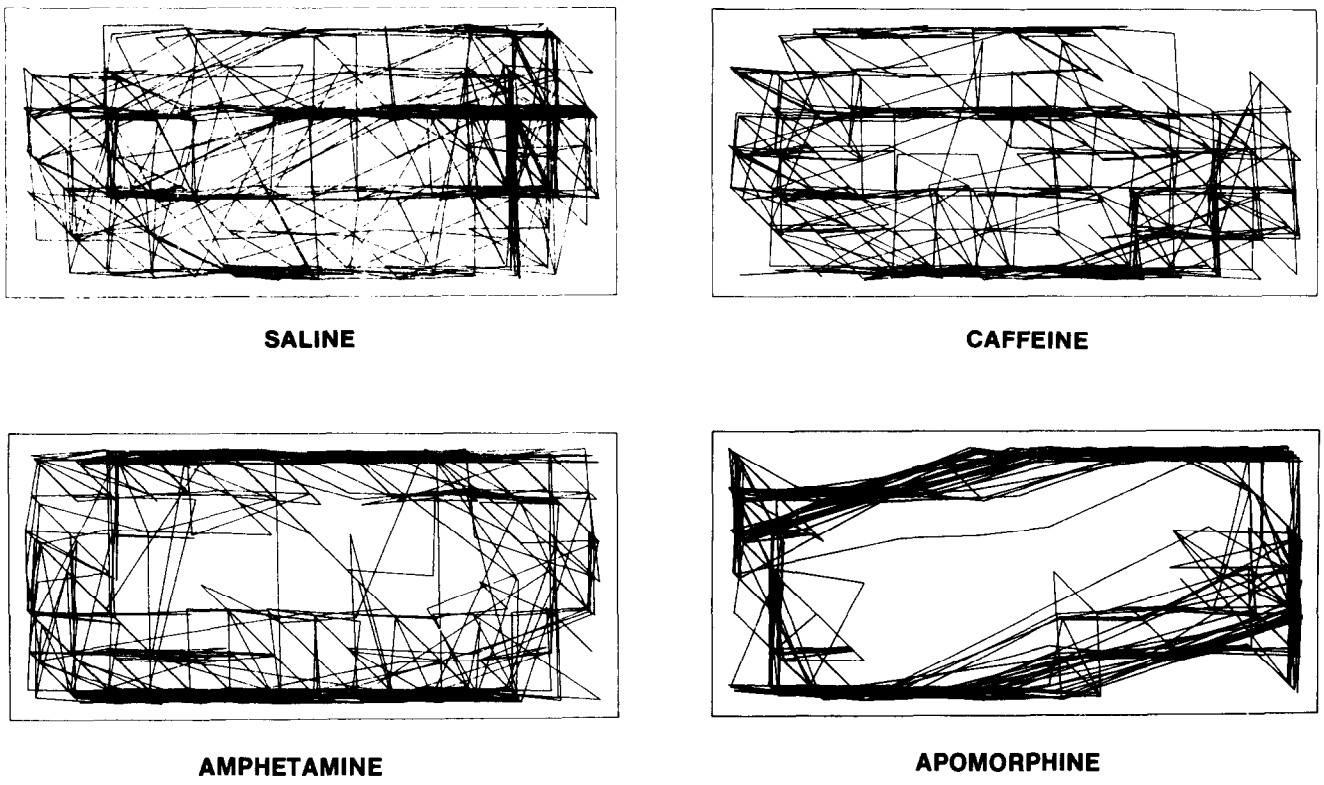

FIG. 9. Spatial patterns of locomotion exhibited by stimulant-treated animals. Shown here are the movement patterns exhibited by representative animals given saline, caffeine, amphetamine, or apomorphine. Each plot represents the initial 40 min of activity in the chamber.

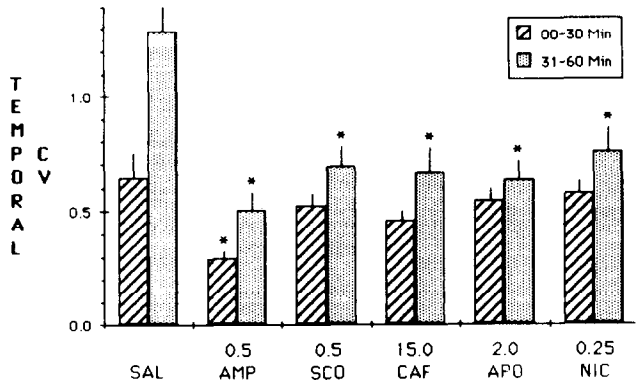

FIG. 10. Effects of stimulants on Temporal CV. Group means for Temporal CV are shown as in Fig. 7. An increase in the Temporal CV reflects a greater tendency for the animal to distribute his time unevenly throughout the 9 regions of the chamber (cf. Fig. 1C). *=Significantly different from corresponding control, $p<0.05$.

spatial patterns of movements made by two untreated rats during successive 10-min blocks of their first exposure to the chamber. As is typical, each rat exhibits a particular pattern of movements which is largely consistent from block-to-block. As described in the Method section, the CV of the distribution of transitions between any of five areas of the chamber was intended as a measure of the degree of redundancy in an animal's spatial patterns of movement. Figure 5 shows the movement patterns of three untreated rats together with the calculated CV and the number of transitions with each. In these examples and in a number of other experiments, we have found no consistent relationship between the amount of locomotor activity and the CV measure of spatial patterning. The Spatial CV consistently reflects the within-session development of progressively more predictable excursion patterns, which is easily recognized when viewing the video displays. In untreated animals, the Spatial CV for the first half of the session is almost always lower than the CV for the second half of the session. The within and between session trends for the Spatial CV measure are shown in Fig. 6 for 14 untreated animals tested in 3 successive hour-long test sessions during the animals' night-time. That the difference between the first and last halves of the session is not simply related to the lesser amount of activity toward the end of the session is confirmed by the observation that some treatments having no effect on the amount or habituation of activity are able to disrupt this trend toward higher Spatial CVs in the last half of the session [1,9].

The distribution of the transitions between the five areas can also be used to assess the degree to which an individual

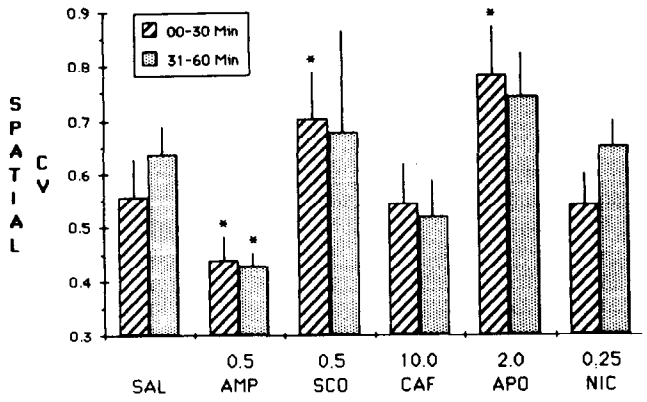

FIG. 11. Effects of stimulants on Spatial CV. Group means for Spatial CV are shown as in Fig. 7. An increase in the Spatial CV reflects a more repetitive pattern of movements. See text for the definition of the Spatial CV. *=Significantly different from corresponding control, $p<0.05$.

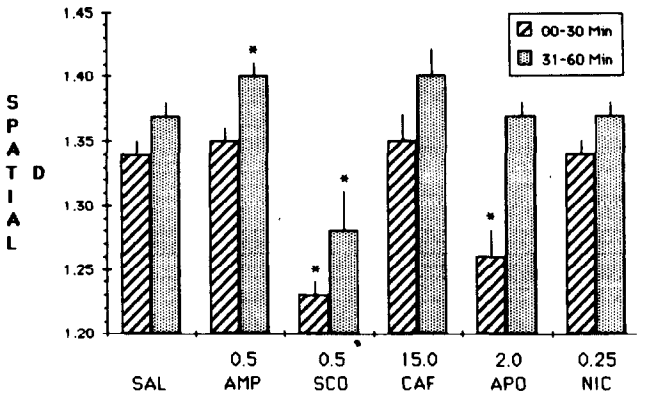

FIG. 12. Effects of stimulants on Spatial D. Group means for Spatial D are shown as in Fig. 7. A decrease in the Spatial D reflects a more smooth pattern of movements. *=Significantly different from corresponding control, $p<0.05$.

animal exhibits the same spatial patterns of movement from one part of a session to another or from one day to another. For example, when an animal follows the same paths in both the first and second halves of the session, as suggested in Fig. 4, highly significant correlations are obtained between the corresponding sets of transition frequencies. For example, in a group of 14 untreated rats tested for an hour in each of three sessions, the median correlation coefficients between the two halves of each session were 0.631, 0.705, and 0.765. Similarly, the median correlations between sessions 1 and 2 or 3 were 0.761 and 0.851, and between 2 and 3 it was 0.700. We have previously demonstrated that some drug treatments significantly disrupt these correlations [1,2]. In some cases, it appears that this measure of the predictability of the animals' spatial patterns of movement is more consistent than is the Spatial CV measure.

Effects of Stimulant Drugs

A variety of drugs having the common characteristic of increasing the amount of locomotor activity have been examined in dose-response studies using the BPM, as described in the Method section. For the present purposes, dose groups for between-drug comparisons were selected to be as similar as possible with respect to the total number of Crossovers during the hour-long test sessions. Even at doses producing comparable levels of behavioral activation, each of the stimulant drugs produces a unique profile of changes in the more descriptive measures of the qualitative aspects of the animals' behavior. As illustrated in Fig. 7, the amount of hyperactivity induced by the selected doses of the various stimulants was significantly above control, but only during the second half of the test session for some of the drugs. Two of the most sensitive measures that begin to discriminate among the various stimulants are the amount of time spent in or the number of entries into the center region of the chamber (cf. Fig. 1C). Amphetamine, caffeine, and nicotine induced dose-related increases in Center Entries, while apomorphine and scopolamine decreased Center Entries in a dose-related manner, particularly during the first half of the session. These effects are illustrated for selected doses in Fig. 8. The latter two drugs produce characteristic patterns of locomotion in which the animals rarely move away from the walls. Figure 9 shows the typical pattern of locomotion exhibited by animals treated with apomorphine at doses of 0.5 mg/kg or greater. Most animals treated with apomorphine run around the perimeter of the chamber consistently in one direction for most of the session. Although scopolamine-treated animals also rotate around the perimeter of the chamber, they frequently change directions and pause enroute to investigate the holes and rear against the wall, responses only rarely seen with apomorphine-treated rats [13].

As displayed in Fig. 10, all the stimulant drugs reduced the Temporal CV during the second half of the test session. This result reflects the fact that the high Temporal CV of the controls during the last half hour is largely attributable to their decreased level of activity. That is, the control animals tend to spend most of their time in a particular corner during the last half of the session. Hence, comparisons on this measure are most valuable with treatments producing comparable levels of activity. Among these stimulants, for example, amphetamine appears to be unique in its ability to reduce the Temporal CV during the first half hour. It also produces the greatest decrease in this measure during the second half hour.

The effects of the various stimulants on the Spatial CV are illustrated in Fig. 11. Only amphetamine significantly decreased the Spatial CV, and it did so throughout the test session. Conversely, both scopolamine and apomorphine produced significant increases in the Spatial CV during the first half hour, which reflect the repetitive patterns of locomotion induced by these drugs, as noted with the video displays. Despite having produced significant increases in the amount of locomotor activity and decreases in the Temporal CV, neither caffeine nor nicotine significantly altered the Spatial CV measure. Similarly, our observations of the movement patterns exhibited by animals treated with either nicotine or caffeine reveal that these animals, like controls, exhibit preferences for one corner of the chamber and predictable excursions from that corner to other parts of the chamber. That is, the structure of their locomotor patterns is largely similar to that exhibited by untreated or saline control animals.

For the same reason, no significant effect was observed on the Spatial D measure with either caffeine or nicotine (Fig. 12). It should be noted that highly varied locomotor paths would be expected to increase the Spatial D while decreasing the Spatial CV. Corroborating the results observed with the Spatial CV measure, the Spatial D measure was significantly increased by the selected dose of amphetamine. This effect was particularly marked in the latter half of the test session, reflecting a progressive increase in the degree of variability in the locomotor paths exhibited by amphetamine-treated rats across the session. Conversely, the highly repetitive circling patterns of locomotion exhibited by animals treated with scopolamine or apomorphine resulted in significant decreases in the Spatial D (Fig. 12), which correspond to the significant increases found with the Spatial CV measure.

TABLE 1

SUMMARY OF SELECTED MEASURES ACROSS ALL FIVE DRUGS

Drug	Temporal CV		Spatial CV		Spatial D		Center Entries	
Amphetamine	↓	↓	↓	↓	–	↑	↑	↑
Scopolamine	–	↓	↑	–	↓	↓	↓	↑
Apomorphine	–	↓	↑	–	↓	–	↓	–
Caffeine	–	↓	–	–	–	–	–	↑
Nicotine	–	↓	–	–	–	–	–	–

The pairs of arrows or dashes represent results for the first and second half hour blocks. With respect to control, (↑) represents a significant increase, (↓) represents a significant decrease, and (–) represents no significant change.

DISCUSSION

The detailed analyses of spontaneous behavior provided by the BPM system have revealed important and consistent features of a rat's responses to a novel environment which are differentially affected by stimulant drugs. The analyses of spatial and temporal patterns of locomotor movements described here indicate that although it is complex, the detailed structure of rat locomotor activity is explicable and amenable to statistical description. One of the most unique aspects of the BPM system used in the present studies is that it provides a permanent computerized record of the raw data, which enables repeated visual displays of the detailed movement patterns and the calculation of multiple complex descriptive statistics. Most other systems which provide some form of graphic representation of movement patterns rely on real-time processing of both graphic displays and descriptive statistics and record in a permanent fashion only summarized data. The approach used here is to collect and store all the raw data at the expense of providing immediate feedback regarding the behavior of the animals. It should be noted, however, that some simple measures of activity could be assessed at the time of data collection.

Perhaps the most instructive use of the raw data with regard to gaining an understanding of the structured manner in which a rat explores the test environment is the generation of reconstructed visual images of the sequences of holepokes, rearings, and locomotor movements on the computer terminal. The flexibility with which the investigator can speed up or slow down this display and the degree to which the displayed information is abstracted greatly facilitate the appreciation of treatment-induced differences in the animals' behavior. In our opinion, based on extensive experience with both this system and videotape recordings of the animals themselves, the computerized displays of abstracted forms of the information are much more efficiently interpreted than are standard video displays. The ability to produce clear images at very high speeds greatly simplifies the task of detecting complex patterns that may evolve slowly throughout an hour test session. Further, the ability to calculate descriptive statistics which may corroborate or contradict the inferences drawn from observations of the visual displays is a major advantage of the BPM system over a videotape system.

The visual displays provided by the BPM have revealed a remarkably consistent structure in the patterns of movement exhibited by untreated rats. The animal's locomotion is organized around a self-selected home area, usually a corner, and excursions from the home area out to various parts of the chamber and back. Each animal consistently adopts the same corner as his home area on successive exposures to the same chamber, and each animal establishes predictable excursion paths (Fig. 4). As a result of the consistency of these patterns, the distributions of transitions between different parts of the chamber are highly correlated within each animal across time either within a session or between sessions. From the current studies of the effects of stimulant drugs, it is clear that some drugs, such as amphetamine, disrupt this normal structure by producing highly varied patterns of directional changes. It should be noted, however, that relatively high doses of amphetamine (e.g., 5.0 mg/kg) often induce stereotyped spatial patterns of locomotion, as first noted by Lat [14] and later demonstrated more systematically by Schiorring [23]. The present results also indicate that other stimulant drugs essentially replace the normal patterns of locomotion with new, even more highly structured patterns. For example, both apomorphine and scopolamine induce movement patterns which are very predictable and seemingly characteristic of each drug. The redundancy in these patterns is reflected in significant increases in the Spatial CV (Fig. 11) and decreases in the Spatial D (Fig. 12). It also appears that stimulants such as caffeine and nicotine do not disrupt the normal structure of the animals' spatial patterns of locomotion. With these drug treatments, it is evident that each animal adopts a preference for a particular home area and establishes preferred excursion routes which are no less predictable than are those of controls. Hence, neither caffeine nor nicotine produces a significant alteration in the Spatial CV measure (Fig. 11).

Both the measures of Spatial CV and Spatial D evaluate spatial pattern, and in the Method and Results sections it was implied that they do so in a reciprocal manner. That is, a drug-induced increase in CV is mirrored by a decrease in D, and vice versa. Figures 11 and 12 are essentially consistent with this suggestion, but there are some exceptions to this rule that reflect the different ways these two measures are derived. The Spatial D assesses the relative smoothness or roughness of the locomotor path, independently of the portion of the chamber traversed. On the other hand, the Spatial CV is sensitive to the consistency with which certain areas are explored relative to others (the basis of the calculation).

Hence, these measures are not equally sensitive to the subtle details of behavior noted by observing the computer reconstructions of the patterns. For example, between the first and second half hour, the Spatial CV increases (Fig. 11),

but a reciprocal decrease in Spatial D is not observed, rather, it also increases (Fig. 12). In the first five min after their initial introduction into the BPM, a control animal typically explores the outer boundaries of the chamber. Thereafter, an animal-specific pattern emerges, becoming more recognisable over the next 30 min or so, with repeated outward excursions restricted to certain routes, followed by returns to the self-selected home area, and then finally fewer excursions punctuated by longer and longer rests in the home area. The effect on Spatial D of the first five min of perimeter exploring is that of a relatively smooth, simple long path (low D). Across time, the paths become less smooth and more complex as shorter movements are exhibited (increased D), even while the path is evolving into a recognisable pattern. In contrast to the Spatial CV, it is feasible to calculate D with 10-min resolution (data not shown), and the lower D seen in the first half hour is in fact restricted to the first 10 min. This measure is therefore very sensitive to short path segments. This sensitivity explains why the effect of amphetamine is to increase D, since the animal has an ill-defined home area if any, and so makes few or no long excursions back to it; rather, short movements with frequent small shifts in direction prevail. However, it is clear that animals treated with apomorphine or scopolamine, who persist in repetitive perimeter circling will have a less complex path with few short segments; hence D will remain low.

Although all five stimulants tested produced significant increases in Crossovers at least in the second half hour, there were three different trends in the pattern of results: (A) a change toward a nonrepetitive, less smooth path, widely distributed locomotion (amphetamine); (B) a change in the opposite direction toward a smoother path with more repetitive movement patterns (scopolamine and apomorphine); and (C) little change observed (caffeine, nicotine). These results are summarized in Table 1.

Amphetamine was most easily differentiated from all other groups, as it was the only drug which caused a decrease in Spatial CV, an increase in Spatial D, or an increase in Center Entries in the first half hour. Scopolamine and apomorphine were readily distinguished from the others by the opposite effect: increase in Spatial CV and decrease in D. In this case, Center Entries were useful in distinguishing the two drugs from each other. Although both drugs decreased this measure in the first half hour, apomorphine produced no change and scopolamine elicited a significant increase in the second half hour. It is noteworthy in this context that apomorphine decreased and scopolamine increased both holepokes and rearings [13]. Finally, as indicated by the video replays, caffeine and nicotine did little to change the normal pattern, minimal changes in these measures were observed. However, once again Center Entries could distinguish between the two drugs, since only caffeine produced a pronounced and significant increase in this measure in the second half hour. As with the difference between scopolamine and apomorphine, caffeine increased and nicotine decreased both holepokes and rearings (data not shown). Hence, the present results indicate that the measures intended to be descriptive of qualitative differences in the behavioral effects of drug manipulations are useful in making distinctions among the effects of a group of drugs having the common effect of increasing the amount of locomotor activity.

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