Behavioral "Spectoscopy" with the Force-Plate Actometer

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Monitoring the behavior of animals is an important tool for screening potential new drugs for both effectiveness and safety. This article describes a unique approach capable of detecting subtle effects that are in many cases not measurable with standard methodology. Pharmaceutical and biotechnology companies must correlate behavior information with chemical data (pharmacology, clinical chemistry and drug metabolism), physiological data (blood pressure, electrocardiograms, EEGs, etc.) and pathology data to arrive at a final conclusion.

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Basic biobehavioral research has relied heavily on behavioral measurements in animals as a key part of the scientific discovery process. Alzheimer's disease, Parkinson's disease, drug abuse, psychiatric disorders (schizophrenia, depression, etc.), analysis of brain circuits, and molecular genetics (e.g., knockout mice), have all relied on rodent modeling efforts that have measured locomotor activity, tremor, stereotypies, and rotational behavior, to name a few of the behaviors that can be quantitated with the force-plate actometer. A force-plate actometer is an instrument that uses a horizontal sheet of stiff lightweight metal supported by four force transducers to measure the attributes of the behavior of an animal that moves around on the horizontal sheet or plate. It measures the distance traveled, as do conventional photobeam actometers, but the force-plate approach allows for a much broader and higher precision look at behavior than the photobeam or video methods. Thousands of papers have been published involving the measurement of these behaviors with disparate types of instruments.

Though it often goes without being said, most scientist would agree that, "Instruments shape research, determine what discoveries are made, and perhaps, even select the types of individuals likely to succeed as scientists." (1) Inherent in the scientific instrument is the capacity to render reliable quantitative measurements. In the behavioral field, this has always been a challenge, yet few have undertaken the task of developing new instruments with a relatively wide range of applications. The need for improved behavioral measurements is acutely illustrated by a recent mouse behavioral genetics study (2) that showed how difficult it is to replicate behavioral measurements across laboratories. The greater temporal and spatial resolution of the force-plate actometer compared to existing instruments should make it possible to achieve quantitative reliability across laboratories studying rodent behavior as an end point.

With the advent of the deciphering of the mouse genome and the dawning of the proteomics era, behavioral measurement as a window into brain function and, therefore, gene and protein function in health and disease, will become ever more important. The force-plate actometer affords the means to extract large quantities of functional information from a single recording session (e.g., distance traveled, number and duration of wall rears, presence and frequency of tremor, gait disturbance, rotations, rhythmicity of stereotypies, and more). As such, this approach to behavioral measurement, when combined with the necessary computer algorithms, will be able to provide for relatively high throughput, multi-dimensional screening for gene manipulations in mice or rats.

Overall, the significance of this instrument is that it will provide for better quantification of behavior than has heretofore been possible, and it will achieve this with a single instrument. Availability of this instrument to life scientists around the world will accelerate preclinical biomedical discovery related to a broad range of central nervous system disorders.

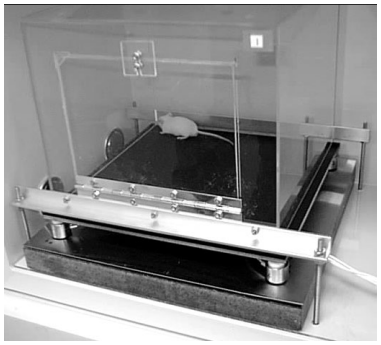
Despite widespread use of photobeam-based systems to measure the effects of drugs, lesions, or gene manipulations in rodents, these de-

vices have limited spatial and temporal resolution for describing behaviors of interest. (A substantial amount of this paper is quoted or paraphrased from ref. 3.) For example, even the "high resolution" systems have beam spacings of about 2.5 cm. When the species of interest is the mouse, this spatial resolution is only one-half a body length, and this quantizing error may make it difficult to detect patterns of behavior more complex than movement from place to place. Another long-standing experiment for which the photobeam apparatus is not well suited is measuring amphetamine-induced stereotypies (4), because stereotypies occur when the rat is not locomoting. In the photobeam apparatus, counting consecutive breaks of the same beam has been claimed to be a measure of stereotypy, but this method depends on the propitious positioning of the rat with respect to a particular photobeam. Therefore, this latter method carries with it a considerable amount of unspecified error that can only be averaged out by using larger numbers of subjects. Consequently, direct observational methods for quantifying amphetamine-induced stereotypies continue to be in widespread use (5,6). Of course, such observational methods are labor intensive and do not lend themselves to quantitation based on frame-by-frame scoring of video recordings by human observers. The force-plate actometer provides for computer scoring of amphetamine-induced stereotypies. The instrument has both spatial and temporal resolution that far exceeds the photobeam methods, and in addition to allowing for machine scoring of amphetamine-induced stereotypies, the instrument can quantify whole-body tremor, rotational behavior, and locomotor activity from a single session of data recorded from either rats or mice. The type of behavioral measurement is determined by the kind of software applied to the raw data.

A force plate actometer is an ensemble of mechanical, electronic, and computing elements that embody mathematical and physical principles to produce measurement of whole-organism behavioral attributes relevant to basic neuroscience research. A force-sensing plate is used to quantify an animal's movements that are confined to a horizontal sensing area by an enclosure suspended a short distance (2 mm) above the upper surface of the force plate. The enclosure may be either square or cylindrical, depending on the aims of the research. In order for the positional information of the animal's movements to be referred to the same coordinate system across different recording instruments, the position of the enclosure must be precisely (+/- 0.5 mm) specified in relation to the force plate. This is accomplished by guide holes that accept the "feet" that support the enclosure (see ***F1***, bolt near lower right corner of photo). A description of the components and their assembly into a functioning instrument will be aided by reviewing the physical principles that serve as the essential concepts upon which the measurements are based.

F2 shows a diagram of the force-plate recording area as viewed from above. The four forces f_1, f_2, f_3, and f_4 correspond to the four corners of the force plate and are permanently defined by rigid physical coupling of four force transducers to the force plate material. The fixed locations of the four forces are given by their coordinate locations (X_1,Y_1), (X_2,Y_2), (X_3,Y_3), and (X_4,Y_4), respectively. In this description of principles, lower case symbols represent variables, and uppercase symbols represent constants. By convention (in the disciplines of mathematics and physics), the four support transducers measuring the four component forces are ordered in a counterclockwise fashion with f_1 representing the force in Quadrant I (the quadrant in Cartesian coordinates where x and y are positive). The position of a force applied to the force plate can be calculated by making use of the four forces sensed at the fixed locations defined by the support points. If a force is applied to the horizontal force plate at a point

F1



A prototype force-plate actometer with a CD-1 mouse on the force-sensing plate. The housings of two of the four force transducers are visible as shiny cylinders underneath the front left and front right corners of the Plexiglas cage that confines the mouse to the recording area. From reference 3.

F2

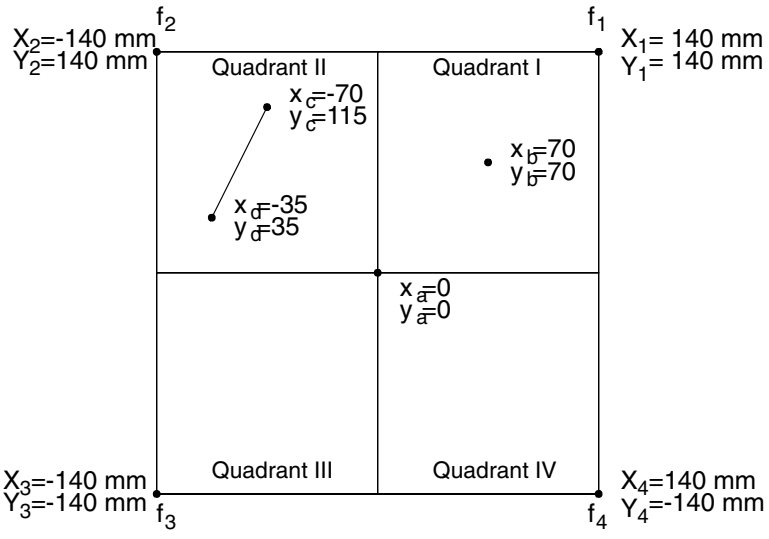


Diagram of the coordinate system defined by the four force transducers. Symbology is explained in the text related to formulas (1) and (2). f_1, f_2,f_3, and f_4 represent the force measures read from the four force transducers that support the plate on which the animal walks. X-Y pairs represent coordinates, with capital letters representing constants and lower case letters representing variables. The enclosure that confines the animal to the plate is designed so the inside corners of enclosure vertically align with coordinates representing the four corners of the force plate (i.e., X_1, Y_1 is the location of corner 1 and the location of transducer 1). From reference 3.

exactly equidistant between each of the four support points (namely, at x_a=0,y_a=0, the center of the coordinate system), then the forces sensed at the four support points will sum to the force in the center. If the applied force is moved along a diagonal from x_a=0,y_a=0 toward (X_1, Y_1), the f_1 force at (X_1,Y_1) will rise and the f_3 force at (X_3,Y_3) at the opposite end of this diagonal will fall in proportion to the distances involved.

An example is shown as a point in the center of Quadrant I in ***F2***. A 100-gram weight placed at point b (x_b,y_b) will result in force measurements of 60g, 15g, 10g and 15g, for f_1, f_2, f_3 and f_4, respectively. The relation between the location of the center of applied force and the four forces at the support points is expressed by these two equations:

$$x = (X_1 f_1 + X_2 f_2 + X_3 f_3 + X_4 f_4) / (f_1 + f_2 + f_3 + f_4) \quad (1)$$

$$y = (Y_1 f_1 + Y_2 f_2 + Y_3 f_3 + Y_4 f_4) / (f_1 + f_2 + f_3 + f_4) \quad (2).$$

Thus, the sum of f_1, f_2, f_3 and f_4 represents the applied force and each of these four separate forces represents the reactive forces supporting the force plate with the load force applied. Different symbols for the applied force and the reactive support forces are not used because physical principles demonstrate that these applied and reactive forces are equal. Equations (1) and (2) provide the basis for calculating the position of the center of force when the individual reactive or support forces are known (i.e., *via* the force transducers). When an animal is the load on the force plate, both the applied force at any instant and the position of the center of force at any instant will vary with time as the animal moves. Provided the measurement of the four forces at the plate support points are taken rapidly enough and the plate and mass of the sensors are low in relation to the applied force, the position of the center of force is independent of the actual applied force. Thus, the position of a mouse on the force plate is defined by the center of force, and the position coordinates become the basis for calculating distance traveled, area covered, spatial pattern, etc. A sample calculation of distance in Cartesian coordinates is as follows: the distance between points c and d (see ***F2***) is:

$$\text{Distance} = ((x_c-x_d)^2 + (y_c-y_d)^2)^{1/2}$$

$$\text{Distance} = ((-70-35)^2 + (115-35)^2)^{1/2}$$

so Distance = $(6125)^{1/2}$ or 78.3 mm.

The force measurements recorded across time (as opposed to the position measurements) serve as the basis for detecting the rhythmicities of the force variations exhibited during walking, jumping, stereotypies, or tremor. The force measurements are for a vertical force vector (F_z) because the force transducers sense vertical forces. The power spectra referred to in the following examples are based on analyses of $F_z(t)$, the vertical force as a function of time.

Measuring Tremor

Tremor is a prominent behavioral end point reflective of CNS dysfunction caused by disease (e.g., Parkinson's disease) or by toxins (e.g., heavy metal intoxication). Despite its importance in neuroscience research, tremor measurement in rodents has often rested on the use of rating scales by human observers; however, sophisticated direct-measurement, instrumental methods have been developed for the specific purpose of quantitating whole-body tremor (7). The force-plate actometer, without modification of its locomotor measurement capabilities, can be used to quantitate whole-body tremor. Inasmuch as harmaline is frequently used as a model of essential tremor observed in the clinical setting (8), this drug was used to demonstrate drug-induced tremor in the C57BL/6 mouse.

C57BL/6 male mice from Charles River Laboratories were maintained under unrestricted feeding and watering conditions. The tremorogenic effects of one dose of 10.0 mg/kg of harmaline hydrochloride were studied when the mice were about 20 weeks old. An intraperitoneal injection of the drug was given about 5 s before the start of a 30 min recording session in the actometer. The recording scheme for purposes of the Fourier analysis of the force signals from the plate was 45 consecutive force time series of 40.96 s in length or 2048 samples at 50 samples/s. Each force datum was the sum of the forces from the four individual transducers that supported the force plate. Exploratory analyses showed that using the sum of the four forces did not distort the spectrum compared to an analysis based on the data from each transducer performed separately. Each of the 45 force-time series was subjected to an FFT (with Hanning data window). The resulting power spectra were examined individually, and the harmaline recording session was compared to a prior saline recording session by ensemble averaging the 45 power spectra separately for the drug and saline sessions.

F3 shows force-time recordings for three frames taken from the harmaline recording session. Frame 1 (40.96 s of recording, beginning 5 s after dosing) shows the force variations during movements around the force plate. In frame 10 (about 405 s after the injection), the first expression of tremor was detected.

It should also be noted that the force variations had subsided to a relatively low level before the tremor was first expressed. This inactivity may be reflecting nervous system changes that precede tremor onset. By Frame 20, the tremor was well developed, but it was not continuous across the entire 40.96-s frame. Power spectra for recording frames 1, 10 and 20 are shown in ***F4*** (top). Harmaline induced a concentration of power in the 12-18 Hz region, and the frequency of the dominant peak

in the spectrum was at a lower frequency in frame 20 compared to frame 10. The reliability and meaning of this latter frequency-shift phenomenon requires further investigation. Power spectra from the entire saline and drug sessions for this mouse are shown in the bottom axes of ***F4***. The predominant peak of the harmaline spectrum was centered at about 13 Hz, and the spectrum for the saline session contained relatively greater power at the lower frequencies (0-5 or 6 Hz) than the spectrum for the drug session.

The force-plate actometer successfully quantitated tremor in the C57BL/6 mouse treated with the tremor-inducing agent harmaline. The attributes of the tremor were consistent with reports in the literature with respect to frequency (9), but the intermittent expression of the tremor may be strain dependent (9). The force-plate approach to tremor measurement was successfully conducted in harmaline-treated rats (10).

Quantifying Focused Stereotypies in Amphetamine-Treated Rats

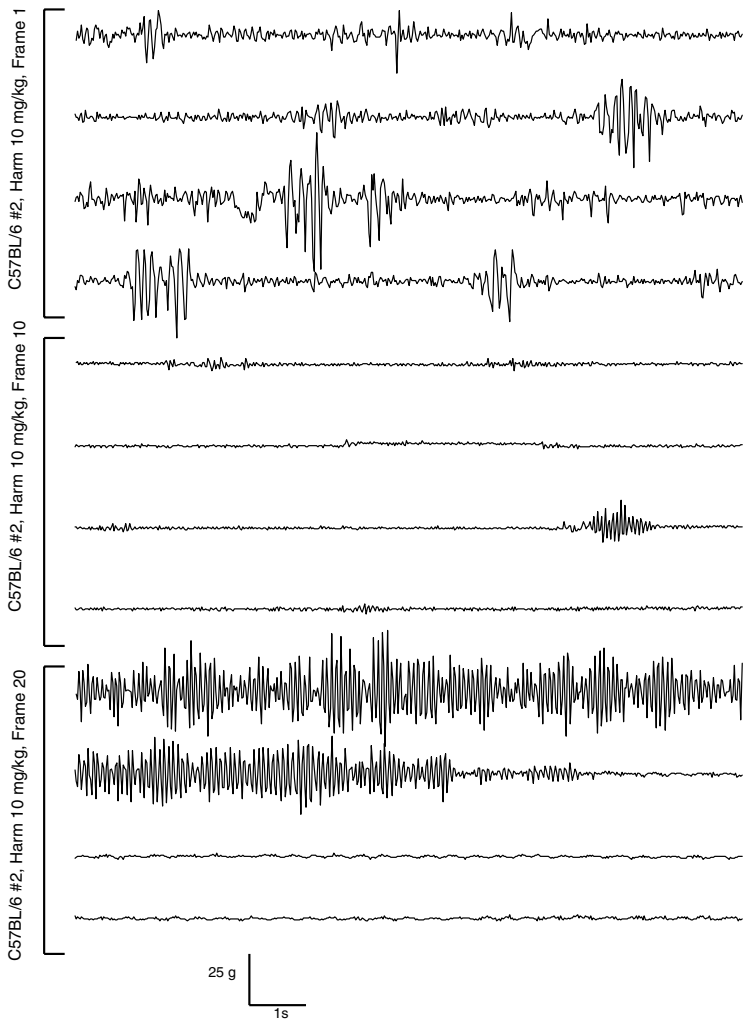
Amphetamine and other drugs that increase the brain action of the neurotransmitter dopamine induce in rats a state of behavioral activation that at low doses produces increases in locomotor activity and at higher doses leads to highly repetitive, rhythmic behaviors such as nose poking, sniffing, head-bobbing and licking, but no locomotion. These high-dose phenomena are collectively referred to as stereotypies (11), and sensitization effects (increasing tendency to engage in stereotypies upon repeated treatment with the same dose) are prominent (6). Amphetamine-induced stereotypies in rats have been used as an animal model of schizophrenia (12) and have figured prominently in theories of basal ganglia function (2,13). Stereotypies in rodents have been scored by human observers who use rating scales. Such methods vary from laboratory to laboratory and are dauntingly labor-intensive. Therefore, objective, instrument-scored stereotypies would be a useful methodology in this area of research. The force plate actometer was used to "machine score" these amphetamine-induced stereotypies with unparalleled temporal and spatial resolution.

Male Sprague-Dawley rats obtained from Harlan Laboratories, and weighing 250 g, were treated with 2.5 mg/kg *d*-amphetamine sulfate once a day according to the schedule previously described (6), which consisted of drug injections on days 1, 2, 3, 4, 5, 6, 7, 9, and 21 over a 21 day period. One-hour sessions were used. Sample data for one rat are shown in ***F5***. The one-hour session was divided into 3-minute time blocks and the power spectra of the force variations ($F_z(t)$) were calculated for each time period (column A in ***F5***).

Column D of ***F5*** shows that amphetamine produced a phase of locomotor stimulation early in the session, but this tendency to move around the chamber gave way to a state of no locomotion in time bocks 7-20. The narrow-band peaks in the power spectra near 10 Hz and the peaks at 2.5 Hz were evident by time block 5 (15 min.). To our knowledge these kinds of data showing that amphetamine induces narrow-band rhythmic behavior near 10 Hz are first time observations. Amphetamine-induced focused stereotypies are characterized by a high degree of rhythmicity, which at this high 10 Hz repetition rate likely reflect underlying neural events operating at the same frequencies. In ***F5***, column A, one can discern a gradual within-session drift in the frequency of the

F3

Time series plots of force variations (F_z) produced by a male C57BL/6 mouse administered 10.0 mg/kg harmaline a few seconds before the recording of frame 1 commenced. In frame 10 the first indication of harmaline tremor within the 30 min. session appeared. By frame 20, tremor was pronounced, but not continuous. From reference 3.



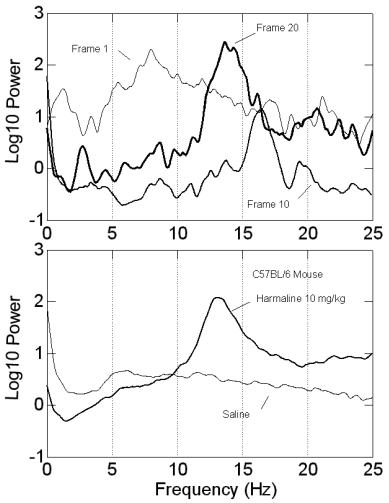
dominant peak in the spectrum from about 10 Hz to about 11 Hz. This frequency shift phenomenon has now been replicated in over a dozen rats. The stereotypy score shown in Column C of ***F5*** was calculated from the power spectra. It was observed that the periods of high locomotion (e.g., time blocks 1 and 2) were accompanied by concentrations of spectral power in the 0-0.5 Hz low frequency band, but when there was no locomotion very little power was detected in this frequency band. At the same time, the power in the peak near 10 Hz increased as locomotion ceased. These observations led to the calculation of the focused stereotypy score plotted in column C of ***F5***. This score is the logarithm of the ratio of the average power in the 8-13 Hz band to the average power in the 0-0.5 Hz frequency band. This score reflects well the combination of intense behavior (primarily head movements) and spatial confinement that are the hallmarks of the focused stereotypy "syndrome" (6). These head movements are often described as head bobbing, rapid lateral head movements or sniffing. Moreover, unlike similar scoring schemes based on human observers using time sampling, our approach to quantifying stereotypies captures all the data with a time resolution sufficient to describe the onset kinetics of the drug. Importantly, the data shown in ***F5*** indicate that in the first 18 minutes of the recording session focused stereotypies are intermixed with some locomotion (see, for example, time blocks 4-6 and compare columns A and D of ***F5***).

It is interesting to note that the distance measure (column B) was relatively constant throughout the session despite the switch from locomotion to focused stereotypies. This means that the distance moved by the center of force (resolved to less than 1 mm) during locomotion was nearly the same as the distance for the focused stereotypies, in which distance was produced by relatively high-frequency, small-amplitude movements of the rat's head. Others (14) have shown that a combination of force and distance measurements taken from a force-plate can be used to estimate energy expenditure of the organism moving on the plate. The implication here is that distance in ***F5*** remained relatively constant across session time because the energy expenditure for focused stereotypies was approximately the same as for locomotion when the animal was affected by 2.5 mg/kg amphetamine. The fact that the rat was not locomoting around the chamber can be shown numerically (but not shown graphically in ***F5***) by calculating the number of low mobility bouts observed during each 3 minute period within the session. A low mobility bout was defined as spatial confinement to a 4 cm diameter circle for 10 s (a maximum of 18 such bouts in a 3-minute period). Applying this criterion to the spatial coordinate data indicated the number of low mobility bouts in the first 3 minutes was 0, and it reached 18 bouts by the 8th time block and remained at 18 bouts through the end of the session.

We have learned from frame-by-frame video analysis and from spectral analysis of spatial coordinates (as opposed to the vertical force variations) that the peaks at 2.5 Hz represent lateral movements of the rat's head during prolonged bouts of

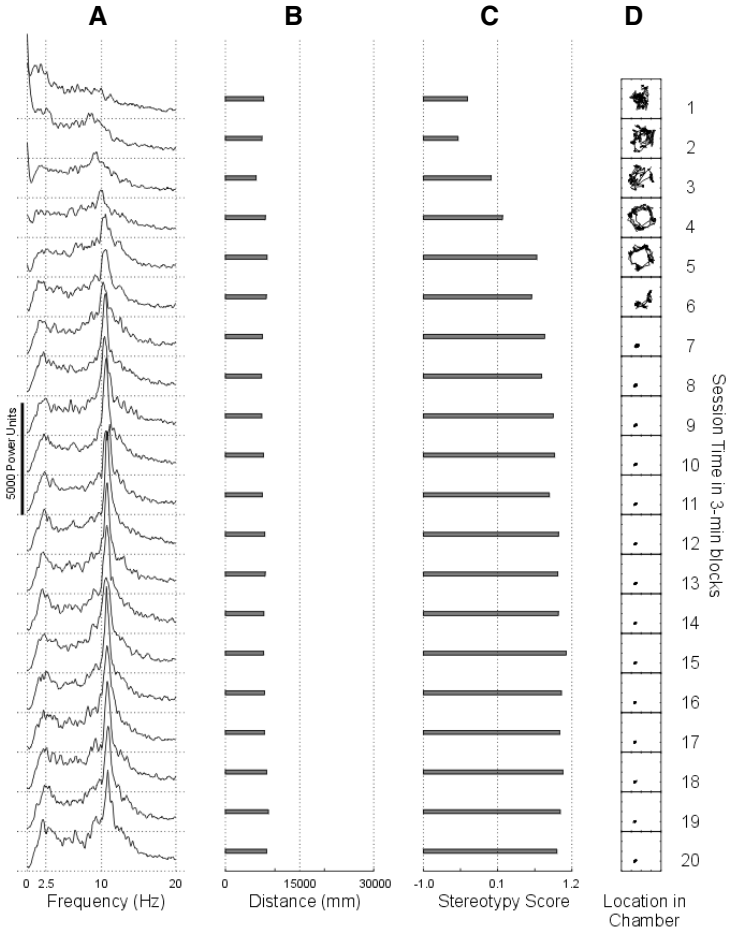
F4

The upper panel shows the power spectra of the time series plotted in **F3**. *In the lower panel are ensemble averaged (across 45 frames of 2048 samples each or 40.96 s) power spectra for the harmaline (10 mg/kg) session and for a saline control session that preceded the drug treatment.*



F5

Power spectra of variations in vertical force (A), distance traveled by the center of force (B), spectrally-based stereotypy score (C), and movement trajectory of the center of force as viewed from above the recording chamber (D) for one rat that received 2.5 mg/kg seconds before being placed in the force-plate actometer. These data are for the 9th experience of the drug treatment that occurred on day 21 of the dosing regimen. Unpublished observations.



"sniffing" and/or "head bobbing" characteristic of focused stereotypy (data not shown for the sake of brevity). Other analyses not shown here indicate that the frequency of the dominant peak shifts to reliably lower frequencies as sensitization to amphetamine's behavioral effect ensues.

The force-plate actometer allowed measurement of amphetamine-induced focused stereotypies without relying on high-labor-cost human observers. In addition, power spectral analysis of the force variations during stereotypy revealed a strong, narrow-band 10 Hz rhythmic component that was present in all rats treated so far with amphetamine in the 2.5 to 5.0 mg/kg dose range. The behavioral methods reported here should provide new tools for exploring the neural substrates of drug-induced rhythmic behaviors. Separating rodent behaviors according to their frequency components is analogous to the separation of molecules in NMR spectroscopy, and this separation analogy informs the title of this article.

Potential uses of the force-plate actometer go beyond the above-described empirical demonstrations. With the proper software it will be possible to detect and quantify wall rears, gait disturbances, jumping, type of spatial patterning of movements, rotations with respect to the center of the chamber, seizure signatures, latency to the first seizure, hyperactivity, hypoactivity, habituation, and energy expenditure over a defined period of time. In addition, this methodology could be combined with existing procedures designed to study learning and memory, such as the radial maze or the operant chamber. In this scheme, the floor of the maze or operant chamber would be a force-plate. Such hybrid procedures would, for example, allow for the simultaneous evaluation of learning capacity and motor behavior and their potential separation. Moreover, in each of these behavioral contexts, the high degree of spatial and temporal resolution of the force-plate methods should make it possible to quantitate assimilation and elimination drug kinetics through the use of behavioral variables.

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