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Automated Measurement of Amphetamine-Induced Focused Stereotypy in Rats and Harmaline-Induced Tremor in Mice: An Introduction to the Force Plate Actimeter

Until now, researchers were required to use a multitude of different behavioral measurement devices for quantification of various behaviors associated with disease models and drug development. This included photo-cell beam boxes to quantify locomotion (1) and rearing activity (2), rotometers to quantify rotational activity (3), and subjective human scoring for stereotypical behaviors (10, 11). In addition, many of these devices work for either rats or mice, but not both species, further compounding the amount of equipment required to quantify behaviors. This article highlights use of the BASi Force Plate Actimeter to objectively quantify amphetamine-induced focused stereotypy in rats, as well as harmaline-induced tremor in mice.

The Force Plate Actimeter (FPA), developed by BASi (Bioanalytical Systems, Inc., West Lafayette, IN) in conjunction with Dr. Stephen C. Fowler (Dept. of Pharmacology and Toxicology, University of Kansas, Lawrence, KS), is designed to be used in a wide variety of applications where the goal is objective quantification of a laboratory animal's behavior with a level of spatial and temporal resolution far beyond that of typical behavioral measurement devices. This device can be used to quantify locomotor activity (4); rotation (1); startle, ataxia (5, 6); focused stereotypies (head bobbing, rearing, etc.) (1, 7); grooming, scratching, and tremor (1, 8). The FPA can measure all of these behaviors in both mice and rats weighing between 15g and 500g.

Direct observational methods for quantifying psychostimulant-induced stereotypies and drug-induced tremor are typically used by researchers (9, 10). These observational methods can be especially difficult in small animals such as mice, where fine movements are difficult to quantify using observer rating methods, and may vary greatly among laboratories in addition to being extremely labor-intensive. Although there are other methods for quantifying tremor, such as measuring changes in current (11) or force on a single strain gauge (12), these methods are unable to measure other behaviors such as locomotor activity, rotational behavior, or ataxia, which can be important in understanding the overall disease model. However, the Force Plate Actimeter has the distinct advantage of quantifying stereotypy and tremor in an objective and automated manner, while also quantifying a multitude of other behaviors of interest to researchers.

Device

The Force Plate Actimeter consists of a force plate supported by four highly sensitive transducers that allow continuous tracking of the animal's position and detection of various behaviors of interest. An enclosure is suspended above the force plate to provide an open-field environment (1756cm^2) in which the animal may move freely (**F1**), and the whole device is enclosed in a ventilated sound-attenuating chamber (13) (**F2**). The Force Plate Actimeter also employs Windows-based software developed to streamline the process of running experiments and analyzing results.

F1. The Force Plate Actimeter consists of a square force plate supported on each corner by a transducer, above which is an open-field enclosure.

F2. The Force Plate Actimeter is enclosed by a ventilated sound-attenuating chamber and uses a proprietary interface to communicate with the FPA software.

Methods

Subjects

Six male Sprague-Dawley rats (278-331g) and eight male CD-1 mice (14-19g) served as subjects. Animals were acclimated to the FPA during a single session and then tested for either amphetamine-induced stereotypy (rats) or harmaline-induced tremor (mice).

Procedures

All recording sessions were conducted sequentially in one FPA enclosed in a sound- and light-attenuating chamber. The interior of the chamber was illuminated by an 8-watt fluorescent bulb located at the top center of the back wall of the chamber.

Rats: Animals were tested over two consecutive days. Half of the animals received saline IP on the first day and 5.0 mg/kg amphetamine IP on the second day, while the other half received 5.0 mg/kg amphetamine IP on the first day and saline IP on the second day of testing. Injections occurred within 5 to 30 sec of the beginning of the testing sessions. Data were collected at a rate of 100 points per second with a moving kernel average of 5 for smoothing (smoothed data not used in spectral analysis).

Mice: Testing consisted of 90-minute recording sessions, preceded by 30-minute acclimation sessions. Half of the animals received 0.03-0.04 ml saline IP and the other half received 16.0 mg/kg harmaline IP. Injections occurred within 5 to 30 seconds of the beginning of testing sessions. Data were collected at a rate of 100 points per second over the course of the experiment.

Procedures involving animals and their care were conducted in conformity with institutional guidelines that are in compliance with national laws and policies (NIH Guide for the Care and Use of Laboratory Animals, NIH Publication N. 85-23, 1985).

Data Analysis

Focused stereotypy score calculations: The force plate is first divided into a 16 by 16 grid of 256 squares. The percentage of time and the force variance while the animal was in each square are calculated. In the above calculations, instances where the animal was in the square for less than one tenth of one second are excluded. The percentage of time spent in each square is multiplied by the force variance for that square and divided by the total number of squares occupied. This value is then summed for all 256 squares to obtain the Focused Stereotypy Score.

Computation of the power spectra: Fast-Fourier transformations (FFT) were performed on force variation in each bout of low mobility (circle radius: 15mm; duration 10.24 sec) to obtain power spectra for rats and across all data in mice. The computed power spectral functions were expressed in terms of magnitude as a function of frequency (spanning a range of 0 to 25 Hz).

Tremor Index Calculations: Data were analyzed in 5-minute epochs. Overall locomotor activity (total distance traveled) and tremor index were calculated for each epoch. To calculate the tremor index, an average power spectrum for each epoch was calculated. The power over the low frequency range (0-5 Hz) was then subtracted from the power over the tremor frequency band (10-15 Hz) to eliminate the baseline spectral power.

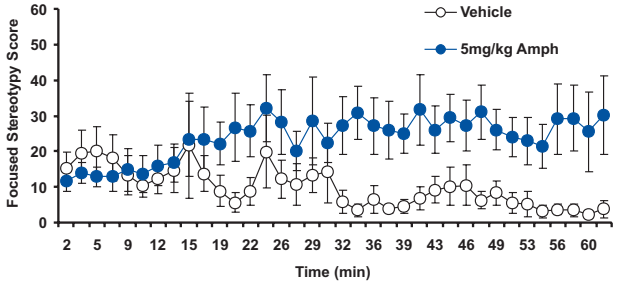
Drugs

Dextro-amphetamine sulfate (Sigma-Aldrich, St. Louis, MO) was dissolved in physiological saline, and injected IP in a volume of 1.0 ml/kg. Harmaline hydrochloride (Sigma-Aldrich, St. Louis, MO) was dissolved in physiological saline and injected IP in a volume of 2.0 ml/kg. Doses are expressed as the salt form of each compound.

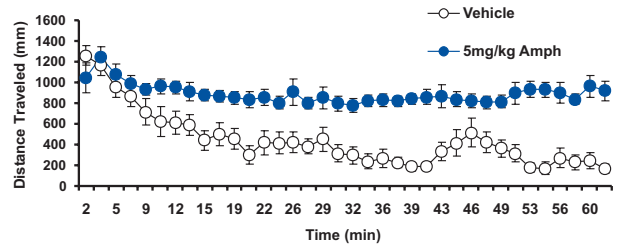
Results and Discussion

Amphetamine-Induced Stereotypy in Rats

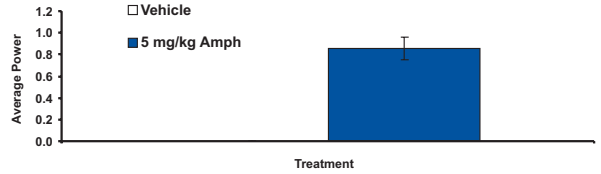
F3. Mean (± SEM) focused stereotypy scores in rats (n=6) following intraperitoneal (IP) vehicle (saline) or 5 mg/kg d-amphetamine (Amph) over time (average of x10, 10.24 sec frames; total of x360 frames = 61.44 min).



F4. Mean (± SEM) distance traveled (mm) in rats (n=6) following intraperitoneal (IP) vehicle (saline) or 5 mg/kg d-amphetamine (Amph) over time (average of x10, 10.24 sec frames; total of x360 frames = 61.44 min).



F5. Mean (± SEM) power in the 9 to 12 Hz frequency range for rat #860 following intraperitoneal (IP) vehicle (saline) or 5 mg/kg d-amphetamine (Amph) for data in each bout of low mobility (BLM). (Average power for vehicle = 0.004±0.001)



Amphetamine began to increase focused stereotypy scores 17.1 minutes after administration and stereotypy scores remained above vehicle levels for the remainder of the 1-hr session (**F3**). Amphetamine also increased distance traveled which became evident at 8.5 minutes post-administration, and distance traveled remained above vehicle levels for the duration of the session (**F4**). In addition, power in the 9 to 12

Hz frequency range (7) was greater following amphetamine compared to vehicle administration, as illustrated by a representative animal in **F5**. Higher doses and/or repeated doses of d-amphetamine may have elicited higher focused stereotypy scores and decreases in distance traveled. However, the present studies utilized an acute, moderate dose of d-amphetamine which produced focused stereotypy and increases in distance traveled. In support of the present findings, amphetamine decreased bouts of low mobility and increased the number of squares utilized on the force plate (data not shown). These findings demonstrate the utility of the Force Plate Actimeter in the automated and objective quantification of stereotypic behaviors in rats.

Harmaline-Induced Tremor in Mice

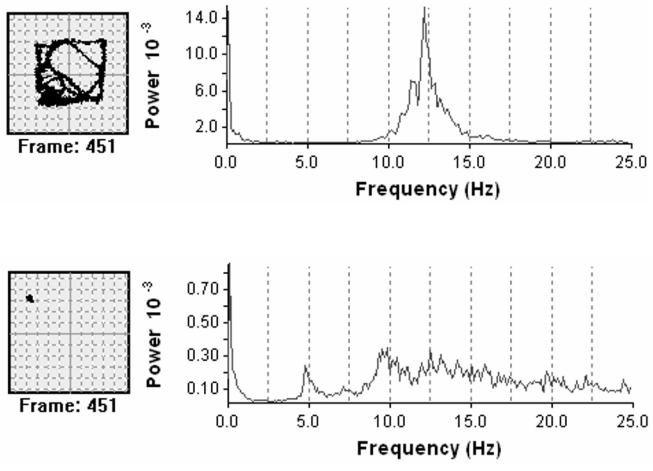
Harmaline caused visible tremor in the mice, which manifested as a strong peak in the 10-15 Hz range (**F6**). The tremor index was higher in the harmaline group for all epochs except the first, and remained high during the full course of the experiment (**F7**).

Locomotor activity in saline-injected mice declined during the first half of the experiment, and then remained essentially constant for the second half (**F8**). In contrast, locomotor activity in harmaline-injected mice increased over time. After the first 30 minutes of testing, activity was significantly higher in harmaline-injected animals compared to saline-injected animals.

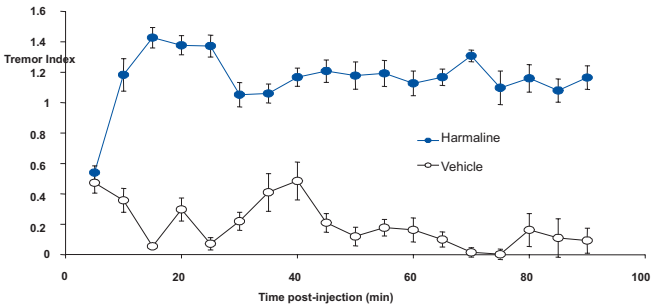
Conclusion

The Force Plate Actimeter can be used to effectively and efficiently quantify focused stereotypy in rats and harmaline in mice, while simultaneously tracking the animal's locomotor activity. The Force Plate Actimeter is a powerful tool for laboratory animal behavior quantification and analysis and can replace a multitude of laboratory instruments previously required to collect comparable data by quantifying multiple behaviors in multiple species using one device.

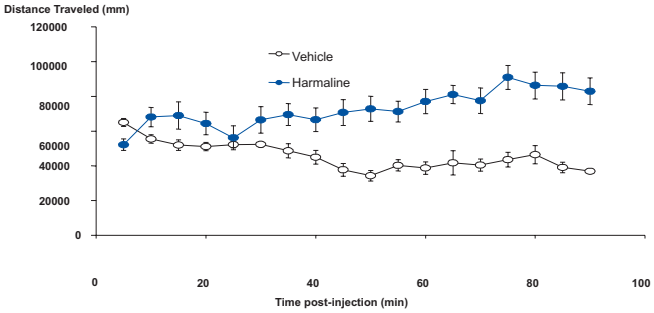
F6. Comparison of average power over frequency band and locomotor activity 75-80 minutes after either harmaline or vehicle (saline) injection in two representative mice, #M250 and #M251, respectively.



F7. Tremor indices after injection of harmaline or vehicle (saline) in mice (n=4).



F8. Locomotor activity after injection of either harmaline or vehicle (saline) in mice (n=4).



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