A Gravimetric Method for the Measurement of Total Spontaneous Activity in Rats (44429)

BRANDON J. BIESIADECKI, PAUL H. BRAND,[1] LAUREN G. KOCH, AND STEVEN L. BRITTON
Department of Physiology and Molecular Medicine, Medical College of Ohio, Toledo, Ohio 43614–5804

Abstract. **Currently available methods for the measurement of spontaneous activity of laboratory animals require expensive, specialized equipment and may not be suitable for use in low light conditions with nocturnal species. We developed a gravimetric method that uses common laboratory equipment to quantify the total spontaneous activity of rats and is suitable for use in the dark. The rat in its home cage is placed on a top-loading electronic balance interfaced to a computer. Movements are recorded by the balance as changes in weight and transmitted to the computer at 10 Hz. Data are analyzed on-line to derive the absolute value of the difference in weight between consecutive samples, and the one-second average of the absolute values is calculated. The averages are written to file for off-line analysis and summed over the desired observation period to provide a measure of total spontaneous activity. The results of *in vitro* experiments demonstrated that: 1) recorded weight changes were not influenced by position of the weight on the bottom of the cage, 2) values recorded from a series of weight changes were not significantly different from the calculated values, 3) the constantly decreasing force exerted by a swinging pendulum placed on the balance was accurately recorded, 4) the measurement of activity was not influenced by the evaporation of a fluid such as urine, and 5) the method can detect differences in the activity of sleeping and waking rats over a 10-min period, as well as during 4-hr intervals recorded during active (night-time) and inactive (daytime) periods. These results demonstrate that this method provides an inexpensive, accurate, and noninvasive method to quantitate the spontaneous activity of small animals.**
[P.S.E.B.M. 1999, Vol 222]

Methods for measuring the spontaneous movement of unrestricted animals without altering the animal's environment are useful in the study of physiology and behavior. Presently available methods for the measurement of animal activity require expensive, specialized equipment. These methods provide measurement of either total activity or a variety of specific patterns of behavior, such as circling or rearing (1). They include the use of photoelectric beam arrays, video tracking, and capacitative detectors (2–7). The photoelectric beam method uses an array of photoelectric beams and detectors that register movement as interruptions of infrared beams. Only movements that interrupt the beams are detected; therefore, the number and position of the beams limit the sensitivity of the method.

The video tracking system transmits the image of an animal from a video camera to a contrast-sensitive tracker that continually records the coordinates of the point of highest contrast on the animal. Movement is registered as the difference between consecutively sampled points, providing improved resolution compared to other methods (6). One disadvantage of this method is that the use of a video camera by its nature requires that the testing occur in some level of light. Light required in this measurement will influence the activity of nocturnal animals during measurements in the dark phase. Capacitative detectors use sensors that measure the change in position of the animal as a change in the electric field of the detector. The sensors create a waveform in which the amplitude is proportional to the mass of the

This work was supported by a grant from the Medical College of Ohio Foundation and the National Institutes of Health (grant #HL 94223 to Dr. Britton).
[1] To whom requests for reprints should be addressed at Department of Physiology and Molecular Medicine, Medical College of Ohio, 3035 Arlington Avenue, Toledo, OH 43614–5804. E-mail: pbrand@mco.edu

Received August 24, 1998. [P.S.E.B.M. 1999, Vol 222]
Accepted May 20, 1999.

0037-9727/99/2221-0065$14.00/0

body and the velocity of movement. An analog-to-digital converter digitizes the waveform, and the digital data are collected by a PC for off-line analysis. This sensor is non-intrusive and can detect movements as fine as bobbing of the head (4).

Activity has also been quantified by direct observation. Direct observation includes any method in which measurement of the data is classified by a human observer (5). Although direct observation requires little or no equipment, it is subject to a greater degree of operator variability than other methods, making it inherently less accurate than other methods. Also, the time required to record a sample makes long-term measurements impractical, and observations cannot be made in the dark.

In the course of studying the endurance capacity of rats, it became necessary for us to measure the total spontaneous activity of rats in their home environment. For this purpose, we developed a method for measuring spontaneous activity that uses the downward force exerted by the rat's activity, recorded as a change in weight on a top-loading electronic balance. Changes in weight can be sampled as digital data, transported to a PC to be recorded, and analyzed off-line. Our method uses commonly available laboratory equipment and allows for a noninvasive measurement of total spontaneous activity over prolonged periods with the rat in its normal environment. Cho (8) has briefly described the use of a scale and kymograph for measurement of activity, and Siviy (9) has mentioned the use of a balance to record the "activity counts" of rats in a metabolic cage. A similar device is also available commercially (MAD-1 Motion/Activity Detector; Sable Systems International, Henderson, NV 89014; sablesys@aol.com). This report describes our approach to the measurement of spontaneous movement in unrestricted rats during both active and quiescent periods.

Materials and Methods

Experimental Set-Up. The basis for this method is that activity produced by a rat will induce a force that can be registered as a change in weight by a balance. The procedure for measuring rat activity consisted of placing a cage containing the rat on the pan of a top-loading electronic balance, taring the balance to zero, and recording the weight changes that occur with the rat's movement. A top-loading electronic balance with a resolution of 0.1 g (model BP 6100; Sartorius, Edgewood, NY) set to acquire data at 10 Hertz (Hz) was used. The dimensions of the balance pan were 18 × 21.5 cm. Rubber pads (2.5 cm^2) were glued to the corners of the balance pan to minimize movement of the rat cage. The cage used was the rat's home cage, a 24 cm × 45.4 cm × 21.2 cm polycarbonate cage with a wire top, provided with normal rat chow and water.

Data Acquisition. The weight registered on the balance, due to the force of the rat's movement, was continuously sampled at 10 Hz and transmitted *via* an RS232 output port from the balance to a Northgate PC (Northgate 386-20-1; Northgate Computer Systems, Plymouth, MN). The data were processed on-line using Labtech Notebook digital acquisition software (Labtech Notebook 7.2.0; Laboratory Technologies Corp., Wilmington, MA). Labtech Notebook was programmed to calculate the absolute value of the first difference (i.e., the absolute value of the difference between consecutive samples:

$$|(x_2 - x_1)|, |(x_3 - x_2)|, |(x_4 - x_3)| \ldots |[(x_n + 1) - (x_n)]|$$

where the x values are the consecutive weights recorded at 10 Hz). Transformation of the data to the first difference removes the influence of changes in total mass on the measure of activity, as occurs, for example, with the evaporation of urine. Absolute values were calculated because downward movements registered positive values whereas upward movement registered negative values on the balance. Converting all changes to positive values allowed summation over time of all weight changes caused by animal movement. The mean of the absolute values was calculated every second (10 samples when sampling at 10 Hz) and written to a file for further off-line analysis. To obtain a single value for total spontaneous activity, the 1-sec means were summed for the entire observation period. Four tests were used to evaluate the accuracy of the method to measure changes in weight.

Evaluation of positional effects. The method was first evaluated to determine if there were differences in the values recorded by the balance when weights were placed in different areas on the bottom of an empty rat cage. The cage bottom was divided into five equally spaced areas; four areas encompassing the four corners, and one area at the center. A 100-g weight was placed randomly and repeatedly in one of the five areas; the total number of trials was 100 (Table I gives the number of trials in each corner and the center). The balance value was recorded, and the average for each area was determined.

Precision of data collection. The system was evaluated to determine if the transmission of data *via* the RS232 port and Labtech Notebook software consistently reflected the changes of weight on the balance. Different "known" weights were added to or removed from the balance, and the output from the calculated "activity" was compared to the

Table I. Effect of Weights Placed in Different Areas on the Balance

Area	Mean ± SEM (g)	Number of trials
Corner 1	199.6 ± 0.05	25
Corner 2	199.5 ± 0.05	19
Corner 3	199.6 ± 0.06	16
Corner 4	199.6 ± 0.05	22
Center	199.6 ± 0.05	18

Note. Data are mean ± SEM.

actual value. At the start, a 50-g weight was placed on the platform, and the balance was tared to zero. Data collection began, and a reading of zero was recorded for 5 sec. Then four different consecutive weight changes were produced: 1) a 100-g weight was placed on the platform for 5 sec, 2) the 100-g weight was removed and zero weight recorded for 5 sec, 3) the 50-g weight was removed so that the balance recorded −50 g for 5 sec; and 4) the 50-g weight returned to the balance so that zero grams were recorded for 5 sec. This sequence was repeated two more times for a single test period of 75 sec. The 75-sec test period was repeated a total of six times to determine the precision of the method.

Evaluation of stereotyped motion. We next tested the ability of the method to quantify movement forces by recording the weight changes produced by the swinging of a pendulum placed on the platform. The pendulum was composed of a 500-g weight suspended by 5 cm of string from a 10-cm horizontal arm that was mounted 27 cm high on a ring stand. The pendulum was placed on the balance platform, the balance tared to zero, the pendulum set in motion, and the resulting forces recorded until the pendulum became motionless.

Evaluation of correction for changes in total mass. We anticipated that during the measurement of spontaneous activity over several hours, with the balance initially tared to zero, evaporation of shed urine and exhaled water would cause a decrease in the baseline weight recorded by the balance. To eliminate any effect of this baseline decrease on the calculation of total spontaneous movement, first differences between consecutive samples were calculated as described above. To determine if this calculation was effective, we mimicked the influence of evaporation by using a syringe pump (model 600-910/920; Harvard Apparatus Co., Inc., Dover, MA) to withdraw water at a constant rate of 5.5 ml/min from a beaker resting on the balance pan while performing the same sequence of addition and removal of weights as used when determining the precision of data collection (see above). [The expected rate of evaporation of urine and insensible water loss of a 300-g rat is 30–40 ml/day, much less than the rate withdrawal of water used in these trials (10)]. The addition and removal of weights was intended to mimic force changes on the balance that might occur due to movement of a rat.

Animal Testing. *Experimental.* Four different measurements of movement were evaluated in two female rats of the Albino Surgery strain housed in the same cage: 1) spontaneous activity recorded for a 10-min period while the rats slept; 2) activity recorded for 10 min while the rats were induced to move with sunflower seeds held above the cage top; 3) activity recorded on three separate days starting at midnight for a 4-hr period; and 4) activity recorded on four separate days starting at noon for a 4-hr period. All recordings were carried out with the two rats in their home cage in the animal facility where the rats were normally housed.

Results

Evaluation of Positional Effects. Table I shows measurement of the same 100-g weight placed randomly a total of 100 times in the four corners and center of the cage. No significant differences were found between the means as evaluated by one-way ANOVA.

Precision of Data Collection. Figure 1A shows the results from sequentially adding or removing weights from the balance. Applied weight changes of 50 g and 100 g appear as peaks rather than steady-state changes because only changes in weight with time were calculated (i.e., differences in weight between consecutive samples). The peaks are all positive because the absolute value of the differences was determined; absolute values were calculated to allow for summation of total activity.

The magnitude of the peaks (approximately 5 or 10 g) is one-tenth the value of the weight changes because the output from the balance was sampled at 10 Hz, and the mean was calculated each second (i.e., the mean of 10 samples was taken). Since the applied changes in weight

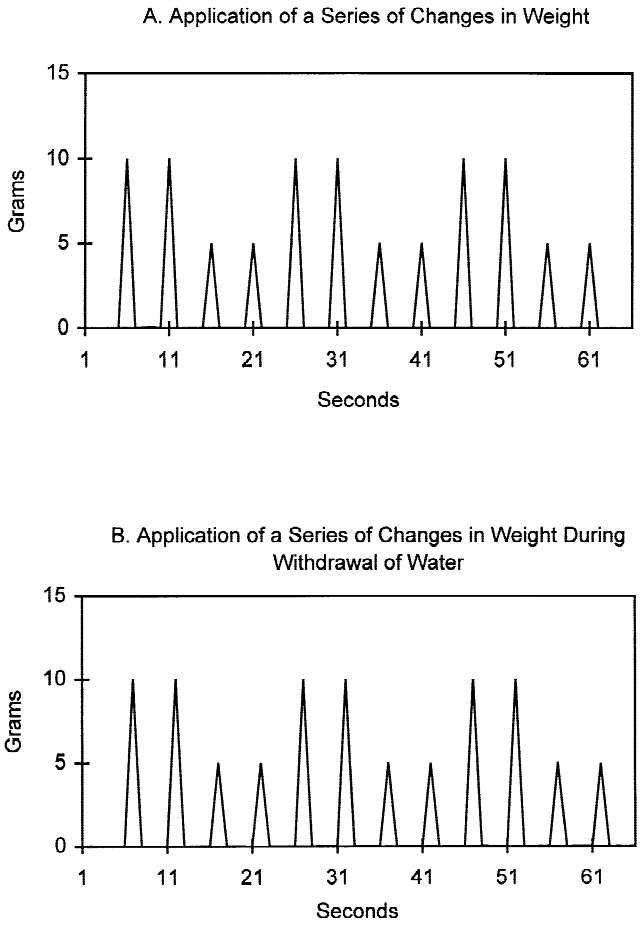

Figure 1. (A) Sequential application of changes in weight as analyzed by calculating 1-sec averages of the absolute value of the difference between consecutive samples of raw data collected at 10 Hz. (B) The same sequence of weight changes were carried out as in Fig. 1A, during continuous withdrawal of water from the cage at 5.5 ml/min.

were recorded within the first 0.1 sec, during the following 0.9 sec a value of zero was recorded (no change in weight). The average of the change in weight sampled in 0.1 sec and the 0.9 sec of zero values yields a number 1/10th the actual change in weight.

To determine the precision of the method, six trials with sequences of known weight changes were carried out. For each trial, six sequential weight changes were induced with 50- and 100-g weights. Keeping in mind that with our method of sampling, weight changes accomplished in 0.1 sec yield values that are 1/10th the actual weight change, the expected sum of the absolute value of the differences in weight was 90 g. The observed mean for six trials of the applied weight changes was 90.1 ± 0.02 g, which was not significantly different from the expected sum. The precision of the method was estimated from the percentage coefficient of variation (% CV; standard deviation/(mean × 100)) for six trials. The % CV for the mean of the six trial sums was 0.06%.

Evaluation of stereotyped motion. Figure 2 shows a recording of the decay in motion recorded from a pendulum placed on the balance. Data shown are 1-sec averages of the absolute value of the first difference obtained at 10 Hz. Fitting a single exponential equation to the data yielded an $R^2 = 0.97$. This exponential decay is the expected result of the constantly decreasing force produced by the diminishing motion of the pendulum on the balance.

Evaluation of correction for changes in total mass. Figure 1B shows that our method of estimating motion is virtually independent of changes in total mass of the rat as could occur with evaporation. The same weight change procedure was performed as shown in Figure 1A, except that water was constantly removed from the cage at 5.5 ml/min to mimic evaporation. The mean value for six trials of weight changes with constant withdrawal of water was 90.7 ± 0.16 g, which was significantly greater than the mean without withdrawal of water (90.1 ± 0.02 g, $p < 0.005$). Although these values are significantly different, the means differ by only 0.6%.

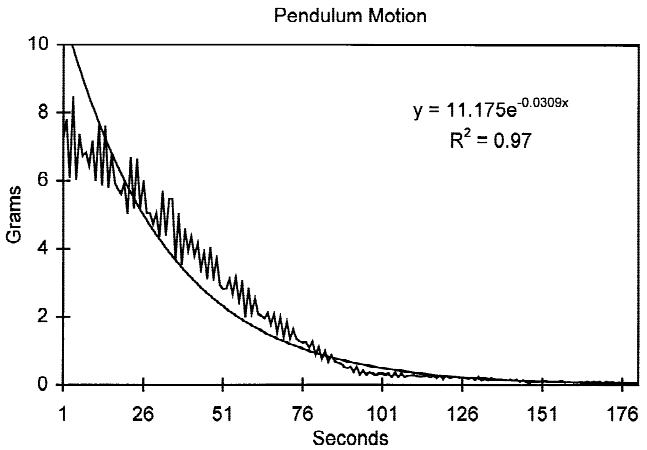

Figure 2. Change in weight over time due to the motion of a pendulum placed on the balance pan. The smoothed line represents a single exponential fit to the data.

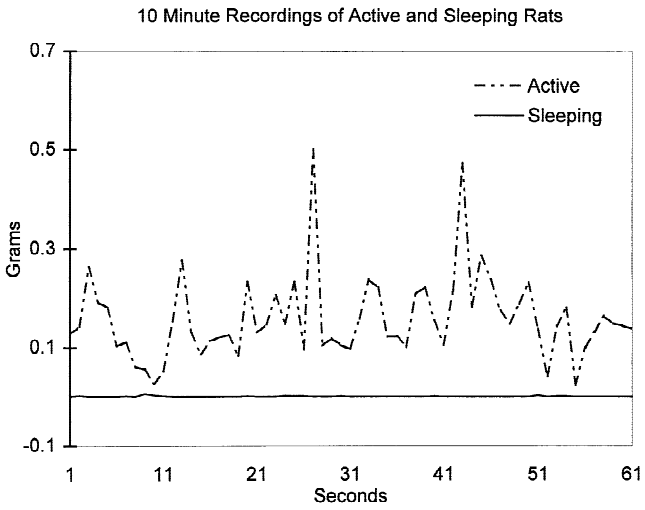

Figure 3. Movement recorded as change in weight over a 10-min period when rats were induced to move (at 1:00 PM, dashed line) and when rats were sleeping (at 1:30 PM, solid line) measured on the same day. Data recorded as 1-sec averages of the absolute value of the difference between consecutive samples of raw data collected at 10 Hz.

Animal testing. Figure 3 summarizes the results of the two 10-min recordings starting at 1:00 PM while the rats were sleeping and at 1:30 PM while the rats were induced to be active. While active, the rats exhibited a total spontaneous activity of 8.8 ± 0.086 g in 10 min (sum of the 1-sec averages), which was significantly different from the sum when they were sleeping (0.025 g ± 0.001, $P < 0.0001$). This test demonstrates the ability of the method to distinguish between active and inactive rats. Figure 4 summarizes the results of the four-hour long recordings ($n = 7$; four records made during the day, and three at night). None of the sums from the daytime recordings overlapped with the sums from the night recordings. The average of the sums of the three daytime measurements, 81.9 g ± 23, was five times less than the average of the sums of the four night-time

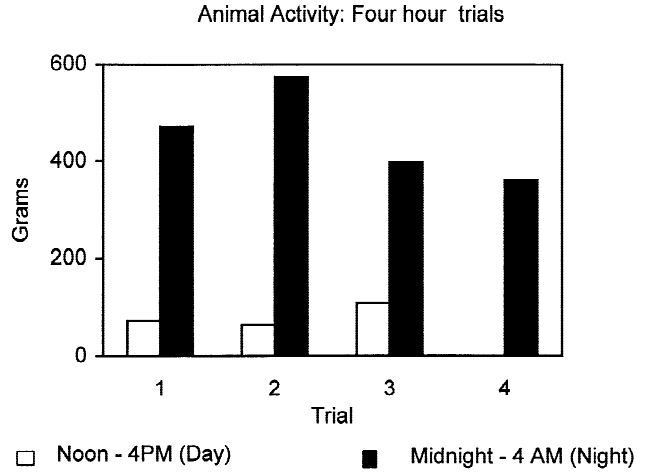

Figure 4. Total activity summed over 4 hr (sum of 1-sec averages of the absolute value of the difference between consecutive samples) from noon to 4:00 PM (day) and midnight to 4:00 AM (night).

measurements, 450.5 g ± 94 ($P < 0.0007$). These results demonstrate the ability of the technique to detect differences between rats exhibiting varying levels of activity and the ability to detect the normal differences in diurnal and nocturnal activity during prolonged recording periods.

Discussion

The method described here provides a simple and reliable procedure for the quantification of total spontaneous activity in the rat. The primary advantage of this method is that the required equipment is readily available in most laboratories and easily assembled. The method can provide both a single value for total spontaneous activity over the measurement period (by summing the activity over a given period) and a measure of the frequency of movement (by counting individual deflections over any desired period). The sensitivity of the method is limited by the resolution of the balance. With a resolution of 0.1 g, movements like grooming and turning the head and upper torso of a rat are recorded as well as rearing and horizontal movements. Thus the method measures total activity. The method requires no observer interaction with the animals. Activity can be recorded for prolonged periods with the animal in its home cage in any lighting condition. This method should be readily adaptable to other species with the limiting parameters being the capacity and resolution of the balance. The main limitation of the method is that all types of movement are recorded as weight changes so different behaviors are not distinguished.

We have shown that this method accurately measures changes in motion. The results are not biased by the position of a weight on the floor of a cage. The method correctly records expected weight changes with a precision of 0.06%. The ability of the method to quantitate movements containing both a horizontal component and a perpendicular component, which more closely represent the movements of animals than static weights, was shown through the pendulum test. The method was also shown to exclude the effect of a loss of total mass such as excreted urine on the total spontaneous activity sum. The differences in the total spontaneous activity sums observed in the various experimental measurements show that the method is able to detect different levels of activity. At night, when rats are most active, they exhibited average activity sums that were 5 1/2 times larger than during the day (Fig. 4). A significant difference in activity was also observed between the same time of sampling on different days. As rat activity is highly variable with time, these differences demonstrate the ability of the method to detect not only differences between active and nonactive states, but also to quantitate different levels of activity.

What our method actually measures is the perpendicular component of the force exerted by a moving rat. That is, any force (F_{total}) applied by the rat to the floor of the cage as it moves may be resolved into two components (11):

$$F_{total} = F\perp + F\parallel$$

where $F\perp$ represents the component of the force that is perpendicular to the plane of the balance pan and $F\parallel$ represents the component of the force that is parallel to the plane of the balance pan. Our method measures $F\perp$, which is related to F_{total} as follows:

$$F\perp = (\cos\theta)\,(F_{total})$$

Cos θ represents the cosine of the angle θ, the angle between the actual direction of the force exerted by the rat and a line perpendicular to the surface of the balance. For forces that are not directly perpendicular, $F\perp$ will be less than F_{total}, and only the perpendicular component will be recorded. Clearly, the perpendicular components are directly related to the total force and thus the total activity. We have no information on the values of θ, which will vary with each movement of the rat. In most conditions the angles of the forces (θ) are not likely to differ systematically between rats so the total activity sum is a valid estimate of spontaneous activity.

In summary, we describe an inexpensive method that provides a simple, precise, and automated procedure to quantitate spontaneous activity in the rat. This method can easily be altered to provide a measure of activity in other species, providing a useful tool for the measurement of spontaneous activity in many different areas of study.

The authors acknowledge the excellent secretarial assistance of Judy Suleski.

1. Ossenkopp K-P, Kavaliers M. Measuring spontaneous locomotor activity in small mammals. In: Ossenkopp K-P, Kavaliers M, Sanberg PR, Eds. Measuring Movement and Locomotion: From Invertebrates to Humans. Austin, TX: RG Landes Company, pp33–59, 1996.
2. Riley H, Spinks A. Biological assessment of tranquillisers. J Pharm Pharmacol **10:**657–671, 1958.
3. Krsiak M, Steinberg H, Stolerman IP. Uses and limitations of photocell activity cages for assessing effects of drugs. Psychopharmacology (Berl) **17:**258–274, 1970.
4. Stoff DM, Dtauderman K, Wyatt RJ. The time and space machine: Continuous measurement of drug-induced behavior patterns in the rat. Psychopharmacology. **80:**319–324, 1983.
5. Robbins TW. Principles of behavioral pharmacology. In: Iverson LL, Ed. Handbook of Psychopharmacology. New York: Plenum Press, Vol **7:**pp37–82, 1977.
6. Vorhees CV, Acuff-Smith KD, Minck DR, Butcher RE. A method for measuring locomotor behavior in rodents: Contrast-sensitive computer-controlled video tracking activity assessment in rats. Neurotoxicol Teratol **14:**43–49, 1992.
7. Sams-Dodd F. Automation of social interaction test by a video-tracking system: Behavioural effects of repeated phencyclidine treatment. J Neurosci Methods **59:**157–167, 1995.
8. Cho MH. Quantitation of spontaneous movements of animals given psychotropic drugs. J Appl Physiol **16:**390–391, 1961.
9. Siviy SM, Kritikos A, Atrens DM. Effects of norepinephrine infused in the paraventricular hypothalamus on energy expenditure in the rat. Brain Res **487:**79–88, 1989.
10. Spector WS, Ed. Handbook of Biological Data. Philadelphia: W. B. Saunders Company, p339, 1956.
11. Hecht E. Physics. Pacific Grove, CA: Brooks/Cole Publishing Company, pp130–134, 1994.