Methodological considerations for measuring spontaneous physical activity in rodents

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Since SPA excludes activity on a running wheel or treadmill (11), SPA has been measured in open-field caging with telemetric implantable transmitters (8), automated infrared photobeams (25), weight displacement with force plates (21, 47), and observer-defined visual (12) and video behavioral analysis (46). Factors such as diet, age, strain/genetics, and sex (42, 44) influence SPA, but more definition is needed. Interpreting results from studies employing different methodology can be difficult. It is unclear whether methodological details, such as contextual cues (e.g., environmental stimuli, location: home cage vs. non-home cage, duration of acclimation period or lack thereof, and use of novel environments) influence SPA measurement and interpretation (5, 37, 45). Few have investigated the possibility that some methodologies and cues could increase measured SPA (13a, 37, 42) or have directly compared different SPA measurement methods (8). Here, we consider the effect of acclimation time, sensory stimulation, vendor, and chamber size on SPA measurement in rodents with varying SPA propensity. We determined which components of SPA (ambulatory and vertical counts, time in SPA, and distance traveled) best describe the variability in SPA measurements. We compare and discuss the interpretation of SPA measurement and exploratory activity measured by infrared photobeams and radiotelemetry. These studies suggest that methods differ in sensitivity for detection of phenotypic differences in SPA and exploratory activity.

METHODS

Animals

C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME), Sprague-Dawley (CD IGS rat CrI:CD strain 001), selectively bred obesity-prone (OP) and selectively bred obesity-resistant (OR) rats (Charles River, Kingston, NY; Charles River, Raleigh, NC; and Taconic Farms, Hudson, NY) were used in these studies. Male mice and rats were used for all studies. Rodents were housed individually with a 12:12-h light-dark light cycle (lights on at 0600) in a temperature-controlled room (21–22°C). Mice and rats in study 6 were housed in solid-bottom cages with corn cob bedding (mice: 8.25" L × 6.25" H and rats: 11.00" W × 16.5" L × 9.0" H). All other rats were housed in single wire-bottom cages (7.0" W × 9.5" L × 7.125" H) with resting platforms available. Mice had nestlets and plastic tunnels, and all rodents had a chewing substrate available (Nylabone, BioServ, Frenchtown, NJ). Rodent chow (Harlan Teklad 8604) and water were allowed ad libitum, except where noted in study 2. All studies were approved by the Institutional Animal Care and Use...
Committee at the Minneapolis VA Health Care System and the University of Minnesota. One group of mice was used, and 11 sets of rats were used for these studies.

### SPA Measurement

SPA was measured using customized, high-precision racks of infrared photoeams (e.g., activity sensors) (Med Associates, St. Albans, VT) placed around a square acrylic chamber (17.00 W × 17.00 L × 12.00 H), as described previously (41). Briefly, for rats, ambulation was detected by two infrared arrays in the x- and y-axes, and vertical movement was detected by a third array at 2 inches above the cage floor. For mice, the third array was 1 inch above the chamber floor. Thus, movement was simultaneously detected in all three axes. From the SPA measurements, time spent ambulating (locomotor activity) and vertical (rearing or standing) was calculated. The sum of time spent ambulating and vertical is referred to as “time spent moving”. In study 4, a second rectangular (10.0 W × 12.0 L × 8.0 H) SPA chamber was used (Columbus Instruments, Columbus, OH) with the Med Associates infrared activity sensors. In study 6, SPA was also measured with radiotelemetry [Data Sciences International (DSI), St. Paul, MN].

### Surgery

Rodents were anesthetized, and a transmitter (TL11M2-F40-EET; DSI) with electroencephalogram (EEG) and electromyogram (EMG) leads was implanted as described previously (22). Bilateral EEG leads were placed 3.1 mm posterior and 1.5 mm lateral to bregma with the incisor bar set at 3.3 mm below ear bars based on stereotaxic coordinates from the rat brain atlas of Paxinos et al. (28). Bilateral EMG leads were secured in the nuchal muscles. The transmitter was placed in a blunt dissected channel along the animal’s back. Animals were fed ad libitum and allowed to recover from surgery for at least 7 days prior to experimental trials.

### EEG-EMG Recording and Physical Activity by Radiotelemetry

EEG/EMG signals from the implanted transmitter were detected by a receiver (PhysioTel RPC-1; DSI) placed beneath the rodent’s home cage, as described previously (22). Electroencephalogram (1.0–30.0-Hz bandpass) and EMG signals (30–100.0-Hz bandpass) from neck musculature were amplified, filtered, digitized, and stored electronically on a computer using the Data Exchange Matrix and DataQuest A.R.T. 4.1 software (DSI). The EEG and EMG signals were downloaded to a PC during the recordings. Spontaneous physical activity was quantified as activity counts when the implanted transmitter moved relative to the receiver that was placed beneath the animal’s home cage. Food and water were available ad libitum.

### Specific Experimental Designs

Experimental designs of five studies are described in Table 1.

#### Study 1: assessment of acclimation time required for SPA measurement

Five-week-old male C57BL/6 mice (n = 28) or 3-mo-old male OR and Sprague-Dawley rats (n = 8/phenotype; Charles River, Kingston, NY) were placed in the SPA chambers without prior exposure to the chambers for three consecutive 24-h periods. Twenty-four-hour SPA was measured daily. Rodents were taken from the chambers 1 h before the next morning to remove feces from the chambers. Food and water were available ad libitum. Time spent moving (sum of ambulatory and vertical movement) was reported.

#### Study 2: determination of complementary and relevant components of SPA

In a separate group of 244 male 6–8-wk-old Sprague-Dawley rats (Charles River), 24-h SPA was measured after a 24-h acclimation period, as described above. Rodents were fed a low-fat diet (D12450B; 10% kcal from fat; Research Diets, New Brunswick, NJ). A portion of this data set is from Refs. 29 and 30.

### Table 1. Study descriptions

<table>
<thead>
<tr>
<th>Study</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Assessment of acclimation time required for SPA measurement&lt;br&gt;Animals: C57BL/6 mice, obesity resistant and Sprague-Dawley rats&lt;br&gt;Main effects: phenotype (OR and Sprague-Dawley) and day (one, two or three)</td>
</tr>
<tr>
<td>2</td>
<td>Assessment of the presence of a sensory attenuation cubicle on SPA&lt;br&gt;Animals: obesity resistant and Sprague-Dawley rats&lt;br&gt;Main effects: phenotype (OR and Sprague-Dawley) and presence of the sensory attenuation cubicle (yes or no)</td>
</tr>
<tr>
<td>3</td>
<td>Effect of chamber size on SPA&lt;br&gt;Animals: obesity resistant and Sprague-Dawley rats&lt;br&gt;Main effects: phenotype (OR and Sprague-Dawley) and chamber size (standard or large)</td>
</tr>
<tr>
<td>4</td>
<td>Effect of vendor on SPA&lt;br&gt;Animals: obesity resistant and Sprague-Dawley rats&lt;br&gt;Main effects: phenotype (OR and Sprague-Dawley) and vendor</td>
</tr>
</tbody>
</table>

### Study 3: assessment of the presence of a sensory attenuation cubicle on SPA

In a separate group of 3-mo-old OR and Sprague-Dawley rats (n = 9–10/phenotype; Charles River), 24-h SPA was measured following a 24-h acclimation period with or without an opaque white sensory attenuation cubicle (MED-OFA-017; Med Associates) placed around the SPA chambers. The multidimension fiberboard sensory attenuation cubicle was illuminated with two lights and ventilated with a fan (28 V), preventing visual cues and dampening auditory and olfactory cues between rodents. According to Med Associates (http://www.med-associates.com), “Sound attenuation levels measured between two cubicles average 44 dB between 100 Hz and 10 kHz and above 10-kHz attenuation increases to 80 dB.”

#### Study 4: effect of chamber size on SPA

In a separate group of 3-mo-old OR and Sprague-Dawley rats (n = 5 or 6/phenotype; Charles River), 24-h SPA was measured following a 24-h acclimation period in two differently sized SPA chambers [“standard size” (10.0 W × 12.0 L × 8.0 H) or “larger size” (17.0 W × 17.0 L × 12.0 H)] without the sensory attenuation cubicle surrounding the SPA chambers. Rodents were fed a low-fat diet (D12450B; 10% kcal from fat; Research Diets, New Brunswick, NJ) without the sensory attenuation cubicle (yes or no).

#### Study 5: effect of vendor on SPA

Three-mo-old male OR and Sprague-Dawley rats (n = 10–12/phenotype) were purchased from two different commercial vendors (Charles River, Kingston, NY and Taconic Farms). Obesity-resistant rats are commercially available from these vendors only. Twenty-four-hour SPA was measured following a 24-h acclimation period without the sensory attenuation cubicle surrounding the SPA chambers.

#### Study 6: assessment of SPA by radiotelemetry and infrared photo-beam analysis

Eight-week-old selectively bred OP and OR rats (n = 6/phenotype; Charles River) were implanted with a radiotransmitter and EEG and EMG electrodes and were allowed to recover for 10 days. Then 24-h physical activity was determined by placing a receiver beneath the rodent’s home cage to detect EEG/EMG signals by radiotelemetry. The EMG signals were digitized, and “activity units” were downloaded from the computer. In four separate sets of OR and OP rats (n = 8–12/phenotype; Charles River), 24-h SPA (Med Associates) was measured after a 24-h acclimation period. SPA measured by radiotelemetry was expressed as activity units, and SPA measured by infrared photobeam analysis, was expressed as time spent moving. In this study, OP rats were used on the basis of the previous data showing that OP and Sprague-Dawley rats had similar SPA (39–41).

#### Study 7: assessment of exploratory behavior and anxiety-like behavior by automatic and manual scoring

Exploratory activity or novel SPA from 3-mo-old rats in study 1 was defined as time spent moving during the 0–30-min period after placing naïve rats into the SPA chamber on day 1. In a separate group of 3-mo-old male selectively bred OR rats and Sprague-Dawley rats (n = 10/phenotype), the light-dark test was performed by placing a dark box insert.
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Fig. 1. Study 1. One day of acclimation is sufficient for C57BL/6J mice, obesity-resistant (OR), and Sprague-Dawley (SD) rats for measurement of 0–24-h spontaneous physical activity (SPA). Time spent in ambulatory and vertical movement in the 0–24 (A and B), light and dark periods (C and D) is shown. *P < 0.001; compared with measurement days 2 and 3 for A, C, and D; B: *P < 0.005, compared with Sprague-Dawley rats on each measurement day. †P < 0.003, compared with Sprague-Dawley rats on measurement days 2 and 3. n = 28 mice; n = 8 OR and Sprague-Dawley rats. Data are expressed as means ± SE.

(ENV-516; Med Associates) into the 17" × 17" SPA chambers (Med Associates). The dark box insert provided an illuminated and dark compartment of equal size (8.5 × 16.75 inches) and an opening (4.5 × 3.5 inches) to allow movement between the compartments. Naïve rats were placed in the light compartment to initiate the 15-min test. The number of entries into the light compartment, into the dark compartment, and total number of entries into either compartment were assessed by two methods concurrently (manually and automatically). Entries were both manually scored by an observer and automatically defined using Med Associates computer software. Manually scored entries were considered valid if two paws (both forelimbs) or if all four paws (forelimbs and hindlimbs) entered into the light or dark compartment. The computer defined an entry as valid when the forelimbs or hindlimbs entered into the light or dark compartment. Hence, when both forelimbs and hindlimbs entered a compartment, the computer scored this as two entries, while the observer defined this as one event. At the end of the 15-min test, rats were returned to their home cage. The SPA chambers and dark box were cleaned with 70% ethanol between tests.

Statistical Analyses

Data were analyzed with Prism 5.0b (GraphPad Software, San Diego, CA) except for study 2, which was analyzed with the free available R statistical software. An alpha level of .05 was used for all statistical tests. Data are expressed as means ± SE. A power analysis was not performed.

Study 1. To determine whether there was an effect of day on SPA, time spent moving on days 1, 2, and 3 were analyzed by repeated-measures ANOVA followed by paired t-tests for mice. For the rats, data were analyzed by two-factor repeated-measures ANOVA with phenotype (OR and Sprague-Dawley) and day (1, 2, or 3) as main effects. When significant main effects were observed, t-tests were performed for data from OR and Sprague-Dawley rats on each day. A one-way ANOVA was used to determine whether SPA differed across days within each phenotype.

Study 2. Different outputs of movement (distance traveled, ambulatory episodes, time spent ambulating, ambulatory counts, time sent rearing, vertical counts, and resting time) were used in principal component analysis with the procop function of the base package in R 2.15. To account for differences in magnitude between variables, data were scaled to unit variance and centered to zero before principal component analysis.

Studies 3–5. Data were analyzed with two-factor repeated-measures ANOVA with phenotype and presence of the sensory attenuation cube (yes or no; study 3), chamber size (standard or large; study 4), or vendor (study 5) as main effects. When significant main effects were observed, differences between phenotypes were determined by t-tests.

Studies 6 and 7. Student’s t-tests were used to determine differences between phenotypes for 24-h total activity counts (study 6), exploratory SPA (study 7), and total number of entries into and time spent within the light and dark compartments (study 7). The coefficient of variation was calculated, and Levêne’s test was used to determine whether the coefficient of variation was significantly different between SPA measured by telemetry and photobeams. A separate test was completed for OR and OP rats. Regression analysis was completed to determine the association between exploratory and habituated SPA on day 2 in rats.

RESULTS

Study 1. Twenty-four hours is a sufficient acclimation period for SPA measurements. There was a main effect of day on time spent moving for mice (24 h: F_{2, 42} = 44.8, P < 0.0001; light period: F_{2, 33} = 150.4, P < 0.0001; dark period: F_{2, 83} = 28.79, P < 0.0001, Fig. 1, A and C). Mice moved significantly more on day 1 compared with days 2 and 3 during the 24-h, light and dark periods (P < 0.0001 for all periods), and there was no significant difference in SPA between days 2 and 3 during all periods (P > 0.05).

For rats, there was a main effect of phenotype and day, but the interaction was not significant (24 h: phenotype: F_{1, 42} = 44.8, P < 0.0001; day: F_{2, 42} = 4.8, P = 0.0134; interaction: F_{2, 42} = 0.04, P = 0.95, Fig. 1, B and D). OR rats moved significantly more during 24-h period than Sprague-Dawley rats on each day (day 1: P = 0.0049; day 2: P = 0.0013; day 3: P = 0.0009). In contrast to OR rats (F_{2, 14} = 2.0, P = 0.17), there was a significant effect of day on SPA in Sprague-Dawley rats (F_{2, 42} = 19.3, P < 0.0001). Sprague-Dawley rats moved significantly more over 24-h on day 1 compared with day 2 (P = 0.0026) and day 3 (P = 0.0023), and SPA on days 2 and 3 was not different (P = 0.53). When the 24-h SPA data
Table 2. Principal component analysis of spontaneous physical activity measures

<table>
<thead>
<tr>
<th>Principal Component 1</th>
<th>Principal Component 2</th>
<th>Principal Component 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Eigenvalue</strong></td>
<td>4.55</td>
<td>1.07</td>
</tr>
<tr>
<td><strong>Relative Importance</strong></td>
<td>0.65</td>
<td>0.15</td>
</tr>
<tr>
<td><strong>Cumulative Proportion</strong></td>
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<td>0.80</td>
</tr>
<tr>
<td>SPA Measurement</td>
<td>Distance travelled</td>
<td>0.457</td>
</tr>
<tr>
<td></td>
<td>Ambulatory Time</td>
<td>0.457</td>
</tr>
<tr>
<td></td>
<td>Ambulatory Counts</td>
<td>0.455</td>
</tr>
<tr>
<td></td>
<td>Ambulatory Episodes</td>
<td>0.417</td>
</tr>
<tr>
<td></td>
<td>Vertical Time</td>
<td>0.143</td>
</tr>
<tr>
<td></td>
<td>Vertical Counts</td>
<td>0.062</td>
</tr>
<tr>
<td></td>
<td>Resting Time</td>
<td>-0.417</td>
</tr>
</tbody>
</table>

were separated into the light and dark periods, there was an effect of day on the light period for OR and Sprague-Dawley rats, respectively (F_{2.33} = 14.2 and F_{2.23} = 19.47, Fig. 1D). During the light period, SPA on day 1 was greater than on days 2 and 3 in both OR and Sprague-Dawley rats (P < 0.0005). In contrast, there was no effect of day during the dark period in OR and Sprague-Dawley rats (F_{2.33} = 14.2 and F_{2.23} = 19.47, Fig. 1D).

**Study 2: Ambulatory and vertical movements describe complementary information.** A principal component decomposition of the data showed the first three principal component vectors account for ~92% of the variance in SPA between rats (Table 2). The loadings of each SPA measurement in these three principal component vectors suggest that all ambulatory measurements (time spent ambulating, distance traveled, ambulatory episodes, and ambulatory counts) describe the same source of variability. All measurements of rearing (time spent vertical and vertical counts) also describe the same source of variability. However, all measures of ambulatory SPA have a higher correlation with principal component vector 1, while the rearing measurements have a higher correlation with the second and third principal component vectors (Table 2). This analysis suggests that vertical and ambulatory measurements of SPA describe complementary, rather than redundant, measurements of activity.

**Study 3. A sensory attenuation cubicle (SAC) dampens SPA in a phenotype-dependent manner.** Two-factor repeated-measures ANOVA indicated a significant interaction between phenotype and the presence of the SAC on time ambulatory (F_{1.17} = 5.5, P = 0.0312), time spent moving (F_{1.17} = 6.8, P = 0.0185), but not time vertical (F_{1.17} = 3.7, P = 0.07) (Fig. 2, A–C). The effect of phenotype and presence of the SAC were not significant on time ambulatory (P = 0.62 and P = 0.68, respectively), time spent moving (P = 0.32 and P = 0.99, respectively), or time vertical (P = 0.31 and P = 0.25, respectively).

Separating data by presence or absence of the SAC revealed that OR rats had significantly greater SPA compared with Sprague-Dawley rats without the SAC (time ambulatory and time spent moving: P < 0.03). In contrast, SPA was similar between phenotypes with the SAC (time ambulatory and time spent moving: P > 0.05). Time spent in vertical movement was not influenced by the SAC in OR (P = 0.11) or Sprague-Dawley rats (P = 0.80).

Paired t-tests revealed that the SAC significantly decreased time ambulatory (P = 0.0332) and time spent moving (P = 0.0459) in OR rats, but the effect of the SAC was not statistically significant for time vertical (P = 0.11). In contrast, the SAC nonsignificantly increased SPA in Sprague-Dawley rats (time ambulatory: P = 0.19; time vertical: P = 0.43; time spent moving: P = 0.16).

**Study 4. Standard-sized testing chambers reduce SPA.** Chamber size and volume had a significant effect on time vertical, and this effect was similar across phenotypes (chamber size: F_{1.10} = 23.47, P = 0.0007; phenotype: F_{1.10} = 0.09, P = 0.77; interaction: F_{1.10} = 3.8, P = 0.10) (Fig. 2, D and E). The smaller, standard chamber significantly reduced time vertical in OR rats (P = 0.0013, Fig. 4B) only (Sprague-Dawley rats: P = 0.14). In contrast, there was no effect of chamber size or volume on time ambulatory despite a main effect of phenotype (chamber size: F_{1.8} = 0.24, P = 0.64; phenotype: F_{1.8} = 11.74, P = 0.0090; interaction: F_{1.8} = 0.5, P = 0.52). Regardless of chamber size, OR rats spent more time ambulating compared with Sprague-Dawley rats, but the difference only reached statistical significance in the larger chamber (large: P = 0.0333 and small: P = 0.08, Fig. 2D).

![Fig. 2. Studies 3 and 4. Twenty-four-hour SPA in OR and SD rats with and without a sensory attenuation cubicle (SAC) surrounding the SPA chamber (A–C). Standard-size SPA chambers reduce vertical movement but not ambulatory movement in OR and SD rats (D and E). *P < 0.05. θP < 0.003 compared with OR rats with the sound attenuation chamber. n = 6–8/phenotype. Please note different y-axes. Data are expressed as means ± SE.](http://ajpregu.physiology.org/)

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Study 5. SPA differs between rats from different vendors. Time spent ambulating in OR and Sprague-Dawley rats differed between rats purchased from Charles River and Taconic Farms (Fig. 3, A–C). There was a main effect of phenotype and vendor on time ambulatory and the interaction was not significant (phenotype: $F_{1,39} = 13.7, P = 0.0001$; vendor: $F_{1,39} = 5.7, P = 0.0216$; interaction: $F_{1,39} = 0.2, P = 0.66$). In contrast, there was no effect of vendor on time vertical or time spent moving despite a main effect of phenotype (time vertical: $F_{1,39} = 0.06, P = 0.85$ and $F_{1,39} = 17.1, P = 0.0002$; time spent moving: $F_{1,39} = 21.2, P < 0.0001$ and $F_{1,39} = 0.3, P = 0.58$). The interaction between vendor and SPA was not significant on time spent vertical or time spent moving ($P = 0.11$ and $P = 0.10$). Interestingly, OR rats from Charles River spent significantly more time ambulating and moving compared with OR rats from Taconic Farms (Fig. 3, A and C). Among Sprague-Dawley rats purchased from different vendors, there was no effect of vendor on time spent ambulatory ($F_{2,35} = 2.9, P = 0.07$), vertical ($F_{2,35} = 0.2, P = 0.98$), or time spent moving ($F_{2,35} = 0.4, P = 0.65$, data not shown).

Study 6. Differences in SPA between OR and OP rats are not observed with radiotelemetry. Spontaneous physical activity measured by radiotelemetry (DSI) indicated that activity units were similar between OP and OR rats ($t = 0.2, P = 0.86$, Fig. 3D). In contrast, SPA measured by photobeam-break analysis (Med Associates) indicated that the same set of OR rats have significantly greater SPA compared with the same set of OP rats ($P < 0.05$, Fig. 3E).

Study 7. OR rats have greater exploratory activity but not anxiety-like behavior, and habituated SPA is associated with exploratory behavior. OR rats had greater exploratory activity compared with Sprague-Dawley rats ($12.3 \pm 1.2$ and $7.7 \pm 1.1$ min, $P = 0.0140$), and exploratory SPA was significantly associated with habituated SPA on day 2 ($r_2 = 0.287, P = 0.032$, Fig. 4A). The light-dark box test indicated that OR rats had significantly more total entries into the light and dark compartments (both observer and computer-defined, Fig. 4, B–D) compared with Sprague-Dawley rats. Entries were similar regardless of whether an entry was defined as two or four paws entering into the compartment (Fig. 4B). The anxiety-like behavior, indicated by total time spent moving in the light and dark during the test, was similar between OR and Sprague-Dawley rats (Fig. 4C). There was significantly more variability in SPA measured by radiotelemetry compared with photo-beams (coefficient of variation for radiotelemetry and photo-beams: 52% and 40%, respectively, $P = 0.0001$).

**DISCUSSION**

As the biological determinants of physical activity are being explored (11, 15), it is important to define methodological factors influencing SPA measurement and whether these factors differentially affect rodents varying in SPA propensity (37, 42). Here, we considered the effect of various methodological factors and contextual cues on measured SPA in rodents with varying propensity for SPA. These data show that a 24-h acclimation period prior to SPA measurements is sufficient for habituation and that ambulatory and vertical measurements of SPA describe different dimensions of the rodent’s SPA behavior. SPA was diminished by reducing testing chamber size and by sensory attenuation. We found that SPA varies between rodents purchased from different vendors and methods differ in sensitivity for detection of phenotypic differences in SPA.

As physical activity measurements are used in obesity, neuropsychology, pharmacology, and toxicology-related research, these data will help to standardize methods and facilitate consistent interpretation of SPA measurements across disciplines. Our results are consistent with others showing that 1) SPA is greater in a novel environment and declines after habituation or acclimation to the testing environment (3); 2) methodological factors related to contextual cues influences physical activity; 3) detection sensitivities for SPA differ between measurement devices (8, 19), and classification of anxiety-like behavior depends on the equipment used (17, 35). These data parallel work showing that the propensity for SPA tracks with the resistance to obesity (16, 27, 38, 39, 41). This is the first report, aside from the mouse phenotype database project (20), to show that the acclimation period, chamber size, vendor, and sensory stimuli have a differential effect on SPA.

Acclimation is a common concern in physical activity studies (1, 13, 14, 26, 31, 37) since environmental novelty independently influences locomotor activity. Locomotor response to a cocaine or saline injection was shown to depend on prior exposure to the testing environment and location (31). Although we show that 24 h is sufficient acclimation period for a 24-h SPA test in C57 mice and Sprague-Dawley rats, the duration of the acclimation period may depend on the test period duration, strain, and/or species. Importantly, our data...

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Fig. 3. Studies 5 and 6. Twenty-four-hour SPA in OR and SD rats purchased from different vendors (A–C). Twenty-four-hour SPA in selectively bred obesity-prone (OP) and OR rats measured by either radiotelemetry or photobeam breaks (D and E). $tP < 0.05$ compared with SD rats. *$P < 0.05$. n = 6–12/phenotype. Please note different y-axes. Data are expressed as means ± SE. Part E is adapted from Kotz et al. (16).
suggest differences in how mice and rats adapt to a new environment. Among the C57BL/6J mice, SPA was greater on measurement day 1 compared with days 2 or 3 during the light and dark period. In contrast, only light period SPA was greater in OR and Sprague-Dawley rats on the first measurement day. These studies suggest that long (i.e., hours) environmental habituation is necessary to eliminate novelty effects and highlight the need of determining adequate habituation duration for a specific test period, strain and/or species.

Rodent locomotor activity has been reported using different measures (34, 38). To test whether different measurements of SPA behavior are redundant, we analyzed different outputs of movement (distance traveled, ambulatory episodes, time spent ambulating, ambulatory counts, time spent rearing, vertical counts, and resting time) from a large data set of 24-h recordings (n = 244 rats) using principal component analysis. Vertical and ambulatory measurements of activity have different loadings to the first two principal component vectors, suggesting they are complementary, rather than redundant measurements of activity. Different measures of vertical movement are redundant, and the same pattern is observed for different measures of ambulatory activity. This analysis indicates that measurements of both ambulatory and vertical activity must be reported during behavioral analysis of rodent SPA, since they describe different dimensions of animal behavior.

Contextual cues and environmental factors influence rodent physical activity (4-6, 24, 43, 45), requiring consideration for design and interpretation of SPA measurements. When we reduced sensory stimuli by placing an opaque white chamber around the SPA chambers, reducing sensory cues (auditory, visual, and olfactory) between adjacent rodents, SPA was diminished. Although sound and illumination are known to influence behavior (5, 23), this is the first report to show lower SPA levels in testing environments with reduced sensory stimuli. It is plausible that sensory stimuli augment activity behavior similar to environmental enrichment strategies (43). Therefore, these data imply that maintaining sensory stimuli between the testing environment and home cage may preserve behavior.

Several apparatus types with wide variability in size are available to measure physical activity, but there is little description of the influence of chamber size and volume on measurements. In our studies, vertical activity was lower in smaller chambers, and this effect was more pronounced in OR rats relative to Sprague-Dawley rats. This difference in SPA is unlikely due to inadequate space for movement since the small SPA chamber was larger than the rodent’s home cage (Table 3).

![Figure 4](http://ajpregu.physiology.org/)  

**Table 3. Size of wire-bottom rodent home cage and SPA testing chambers**

<table>
<thead>
<tr>
<th>Cage</th>
<th>Area, inches²</th>
<th>Height, inches</th>
<th>Volume, inches³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Home cage</td>
<td>66.6</td>
<td>7.125</td>
<td>474</td>
</tr>
<tr>
<td>Standard test chamber</td>
<td>120</td>
<td>8</td>
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<td>12</td>
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<td>Large/standard</td>
<td>2.4</td>
<td>1.5</td>
<td>3.6</td>
</tr>
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</table>

Fig. 4. **Study 7. A:** exploratory/novel SPA is correlated with habituated SPA in OR and SD rats. OR rats have more entries during the light-dark box test; however, total time spent in the light and dark is similar between phenotypes. Entries into the light or dark compartment defined by an observer (B) or the Med Associates computer software (C). **D:** time spent in the light and dark compartments defined by the Med Associates computer software. n = 8–10 OR and Sprague-Dawley rats. Data are expressed as means ± SE. *P < 0.05, **P < 0.005, and ***P < 0.0005.
a size classified as sufficient for rodents (25a). Both the area and volume differed between the standard and larger SPA chamber (Table 3), but despite the twofold area difference, ambulatory activity was not different, and only vertical activity was affected. It is unclear whether the reduction in vertical activity was due to the difference in height or volume. We have previously shown that vertical activity is consistently higher in OR rats relative to Sprague-Dawley rats, and the magnitude of the difference between phenotypes declines with increasing age (39). Rodents in former and current studies were housed in home cages with a height of 7.125 inches, which likely the smaller SPA chambers, prevents rodents from rearing fully. Hence, it is plausible that this may have contributed to lower vertical movement in the small SPA chambers, specifically among OR rats, which are known to have high vertical activity.

We determined whether the magnitude of SPA differed between rodents of varying SPA propensity, since OR rats are commercially available from Charles River and Taconic Farms. The data show that Charles River-OR rats had greater SPA than Taconic Farms-OR rats, while SPA was similar between the Sprague-Dawley rats from different vendors. These data are consistent with our past reports showing greater SPA in OR rats relative to Sprague-Dawley rats from Charles River (16, 39, 41), but the magnitude of difference between OR and Sprague-Dawley rats was lower in rats from Taconic Farms. Breeding generation and breeding protocol may have contributed to OR rat SPA differences between vendors. We previously found high variability in the magnitude of difference in SPA across various groups of OR and Sprague-Dawley rats purchased from Charles River (16). Hence, it is plausible that Charles River rodents, the magnitude of difference in SPA between OR and Sprague-Dawley rats from Taconic Farms varies, and the group that we tested was simply on the lower end for SPA. Additional testing would be necessary to verify this.

Differences in SPA between OR and Sprague-Dawley rats were observed when measured with the photobeam system (Med Associates) but not with a radiotelemetry system (DSI). Differences in detection sensitivities across methods have been noted (19), but it is also possible that radiotelemetry and photobeam systems detect different types of movement. In the radiotelemetry method, EMG leads were placed in the neck musculature because sleep/wake behavior was the primary endpoint; therefore, the activity counts represented head or stereotypic movement. In contrast, ambulation and vertical movements were detected by the photobeam system. We found stereotypic counts were not different between OR and Sprague-Dawley rats despite clear differences in ambulatory and vertical movement (40). Therefore, measuring SPA with EMG leads in hindlimb musculature would be better at detecting large movements (8) and then may provide comparable SPA measurements to photobeam systems. Testing SPA with radiotelemetry and photobeams concurrently in a single group of animals in response to various SPA-stimulating agents would be more definitive.

Exploratory SPA was positively associated with habituated SPA (Fig. 6A), which is consistent with OR rats having greater exploratory activity (18) and initiating more ambulatory episodes (40). To test whether a greater propensity for movement parallels a greater affinity for exploration in OR rats or whether exploratory activity was a marker for anxiety-like behavior (10), we tested anxiety-like behavior with a second test. The light-dark box test (9) exploits the rodent’s natural affinity for the dark and to explore novel environments. Thus, spending more time in the dark and fewer crossings between the dark and illuminated compartments would be considered anxious behavior. We found time spent in the light and dark compartments was equal between the OR and Sprague-Dawley rats, even though OR rats had more crossings between compartments. Hence OR rats appear to have greater activity propensity that is not due to greater anxiety. Others have shown that anxiety-like behavior can vary according to testing methodology, so a battery of tests for anxiety-like behavior may be more conclusive (32, 37).

**Perspectives and Significance**

These data substantiate that testing environment and procedural cues influence physical activity behavior (1). The results underscore the effect of methodology on SPA measurements based on SPA propensity, since clear differences were observed between OR and Sprague-Dawley rats. It is, therefore, likely that central factors differ between rodents varying in SPA propensity, and there is an interaction between those cues and the environment. These studies support efforts to standardize SPA methodology, prevent false-negatives or positives, improve data interpretation, and increase within- and cross-disciplinary comparison between studies reporting SPA.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


