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Stabilization of the wheel running phenotype in mice☆

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HIGHLIGHTS

- Stabilization of the wheel running phenotype in C57BL/6j mice is assessed.
- Male mice exhibit phenotypic stability after nine days of wheel running exposure.
- Female mice exhibit phenotypic stability after eight days of wheel running exposure.
- Distance and speed are adjusted during stabilization, duration remains constant.

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ABSTRACT

Purpose: Increased physical activity is well known to improve health and wellness by modifying the risks for many chronic diseases. Rodent wheel running behavior is a beneficial surrogate model to evaluate the biology of daily physical activity in humans. Upon initial exposure to a running wheel, individual mice differentially respond to the experience, which confounds the normal activity patterns exhibited in this otherwise repeatable phenotype. To promote phenotypic stability, a minimum seven-day (or greater) acclimation period is utilized. Although phenotypic stabilization is achieved during this 7-day period, data to support acclimation periods of this length are not currently available in the literature. The purpose of this project is to evaluate the wheel running response in C57BL/6j mice immediately following exposure to a running wheel.

Methods: Twenty-eight male and thirty female C57BL/6j mice (Jackson Laboratory, Bar Harbor, ME) were acquired at eight weeks of age and were housed individually with free access to running wheels. Wheel running distance (km), duration (min), and speed ($m \cdot min^{-1}$) were measured daily for fourteen days following initial housing. One-way ANOVAs were used to evaluate day-to-day differences in each wheel running character. Limits of agreement and mean difference statistics were calculated between days 1–13 (acclimating) and day 14 (acclimated) to assess day-to-day agreement between each parameter.

Results: Wheel running distance (males: $F = 5.653$, $p = 2.14 \times 10^{-9}$; females: $F = 8.217$, $p = 1.20 \times 10^{-14}$), duration (males: $F = 2.613$, $p = 0.001$; females: $F = 4.529$, $p = 3.28 \times 10^{-7}$), and speed (males: $F = 7.803$, $p = 1.22 \times 10^{-13}$; females: $F = 13.140$, $p = 2.00 \times 10^{-16}$) exhibited day-to-day differences. Tukey's HSD *post-hoc* testing indicated differences between early (males: days 1–3; females: days 1–6) and later (males: days >3; females: days >6) wheel running periods in distance and speed. Duration only exhibited an anomalous difference between wheel running on day 13 and wheel running on days 1 through 4 in males. In females, duration exhibited anomalous differences due to abnormally depressed wheel running on day 6 and abnormally elevated wheel running on day 14. Limits of agreement and mean difference statistics indicated stable phenotypic variability with an up-trending daily mean for distance and speed that stabilized within the first three days in males and within eight days in females. Duration exhibited stable variability after nine days in males and after seven days in females.

Conclusion: Although it is common practice to allow a prolonged (≥seven day) acclimation period prior to recording wheel running data, the current study suggests that phenotypic stabilization of all three indices is achieved at different times with distance and speed exhibiting stability by day three in males and day eight in females. Duration exhibits stability by day nine in males and day seven in females.

☆ All authors aided in animal husbandry, completed data collection and analysis, and prepared the manuscript.

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1. Introduction

The prevalence of hypokinetic diseases—diseases associated with habitually low levels of physical activity including obesity, hypertension, certain types of cancer, diabetes, and cardiac diseases—have remained at epidemic levels in the human population for many years [1]. The understanding of physical activity biology in humans is necessarily limited by both ethical restrictions and developing activity quantification technologies. Researchers have subverted these restrictions by turning to model organisms for activity assessments that relate to the innate molecular and cellular mechanisms that drive daily physical activity patterns [2,3].

The house mouse (*Mus musculus*) and the related laboratory substrains have been shown to exhibit positive characteristics relating to the genetics, physiology, and neurobiology of physical activity [4–12]. The house mouse is also a model laboratory subject due to its high breeding capacity, low animal husbandry maintenance requirements, and general availability from stringent breeders. Furthermore, the house mouse has been shown to exhibit physical activity characteristics similar to humans. Meijer and Robbers [13] demonstrated that when provided access to running wheels in size-biased exclusionary cages, wild mice frequently accessed the running wheel and developed habitual patterns of utilization. The authors concluded that wild mice exhibit a drive for voluntary participation in activity that is not due to stereotypical behaviors such as boredom, anxiety, or seeking food.

The positive characteristics demonstrated in mouse voluntary physical activity in both free-living and captive environments lend well to the use of inbred house mouse strains as a surrogate model of human voluntary physical activity. To date, only a single study has quantified the repeatability of this complex phenotype in laboratory mice after phenotypic stability was achieved [14]. Knab et al. [14] demonstrated preservation of day-to-day phenotypic stability in mice for distance, duration, and speed of wheel running. The initial point of phenotype stabilization after introduction to a novel cage environment and running wheels is yet to be quantified. Typically, after mice are received by a research facility, the animals undergo a quarantine period followed by an initial wheel running exposure period before official data collection begins. These initial phases may consume up to 14 days—longer in some instances—resulting in delayed productivity, elevated husbandry costs, and aging mice. The purpose of this project was to evaluate the time required to achieve phenotypic stabilization of wheel running distance, duration, and speed immediately following animal arrival at a typical mouse husbandry and research facility. We hypothesized that a delay in phenotypic stability in the wheel running response would manifest as depressed mean values and elevated variability values in distance, duration, and speed in both male and female mice.

2. Methods and materials

Twenty-eight male and thirty female C57BL6/j mice (Jackson Laboratory, Bar Harbor, ME), acquired at 8 weeks of age, were immediately housed with free access to running wheels. Twenty-six male and twenty-nine female mice completed the full study. All mice were housed with *ad libitum* access to standard rodent chow (2018 Teklad Global 18% Protein Irradiated Rodent Diet, Harlan Laboratories, Madison, WI) and purified water in standard rat sized cages equipped with mouse lids and filter tops. Solid surface running wheels (450 mm circumference; 70 mm wide running surface) were maintained free of debris, lubricated as needed, and interfaced with calibrated cycling computers (BC500, Sigma Sport, Batavia, IL) to track daily running distance (km) and duration (min). Speed data were calculated as distance per unit time ($m{\cdot}min^{-1}$). Wheel running data were collected every 24 h for 14 days following initial exposure. Following data collection, wheel running data were analyzed statistically by individual one-way analysis of variance (ANOVA) tests followed by Tukey's HSD *post-hoc* analysis to identify specific day versus day differences. An *a priori* alpha value of 0.05 was deemed significant for all statistical calculations. All statistical calculations were computed in the statistical program R (Version 3.2.1).

A Bland–Altman analysis [15] was conducted to calculate mean ± SD differences, limits of agreement, and range of agreement between acclimating (days 1 to 13) and acclimated (day 14) wheel running for each wheel running characteristic. Wheel running data from acclimated (day 14) wheel running was used to calculate differences for each individual mouse across all thirteen acclimating days (days 1 to 13) for each wheel running characteristic. For example, the difference in distance run by one mouse between day 1 and day 14 was calculated as follows:

$$Distance_{day\ 1} - Distance_{day\ 14}.$$

Means and standard deviations (SD) for each day-to-day comparison (day 1 to 14; day 2 to 14; day 3 to 14, etc.) were calculated from the difference values. Both positive and negative limits of agreement (LOA) were calculated for each comparison to represent a 95% confidence interval. The positive LOA were calculated as mean difference + 1.96 ∗ SD; negative LOA were calculated as mean difference − 1.96 ∗ SD. A range of agreement for each comparison was calculated as the arithmetic range of the absolute values of the positive and negative LOA. Lastly, Pearson's correlation (r) values were determined to identify up- or downtrends in the daily mean difference values—day-to-day comparisons (x value) versus mean difference (y value)—and daily within group variability— day-to-day comparisons (x value) versus range of agreement (y value)—for each wheel running characteristic.

3. Results

3.1. Males

One-way ANOVA tests for each wheel running characteristic indicated that day-to-day differences existed—distance ($F = 5.653$, $p = 2.14 \times 10^{-9}$), duration ($F = 2.613$, $p = 0.001$), and speed ($F = 7.803$, $p = 1.22 \times 10^{-13}$). The average daily wheel running distances, durations, and speeds are depicted in Figs. 1–3. Significance tables are also included in Figs. 1–3 to mark the pair-wise comparisons that reached statistical significance. Results from the Bland–Altman (non-graphical) analyses to determine agreement between day-to-day wheel running distance, duration, and speed during acclimation (days 1 to 13) and after stability was achieved (day 14) are shown in Tables 1–3.

The mean difference in day-to-day wheel running distance exhibited a significant up-trend ($r = 0.89$, 95% CI = 0.66 to 0.96, $p = 0.00005$) as the study approached day 14 indicating day-to-day means were becoming increasingly homogenous as the study progressed. Range of agreement data for day-to-day distance exhibited a moderate down trending with dispersed confidence intervals ($r = -0.63$, 95% CI = −0.88 to −0.12, $p = 0.02$) indicating that within group variability was moderately stable throughout the study. Day-to-day differences in mean duration exhibited a moderate uptrend with moderately dispersed confidence intervals ($r = 0.83$, 95% CI = 0.51 to 0.95, $p = 0.0005$) indicating a limited trend toward an increasing mean as the experiment progressed. Within group variation as measured by day-to-day range of agreement showed a significantly decreasing trend ($r = -0.92$, 95% CI = −0.98 to −0.75, $p = 0.000009$) indicating that variability in wheel running duration stabilized over a prolonged period of time. Mean differences in day-to-day speed exhibited a moderate uptrend and moderately dispersed 95% confidence intervals ($r = 0.86$, 95% CI = 0.60 to 0.96, $p = 0.0001$) indicating a limited trend toward increased daily means with experimental progression. Range of agreement data for wheel running speed showed a very low correlation through experimental progression and very dispersed confidence intervals ($r = -0.35$, 95% CI = −0.76 to 0.25, $p = 0.24$) indicating within group variation was immediately stable for this wheel running characteristic.