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Voluntary Wheel Running in Mice

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Abstract

Voluntary wheel running in the mouse is used to assess physical performance and endurance and to model exercise training as a way to enhance health. Wheel running is a voluntary activity in contrast to other experimental exercise models in mice, which rely on aversive stimuli to force active movement. The basic protocol consists of allowing mice to run freely on the open surface of a slanted plastic saucer-shaped wheel placed inside a standard mouse cage. Rotations are electronically transmitted to a USB hub so that frequency and rate of running can be captured to a software program for data storage and analysis for variable time periods. Mice are individually housed so that accurate recordings can be made for each animal. Factors such as mouse strain, gender, age, and individual motivation, which affect running activity, must be considered in the design of experiments using voluntary wheel running.

Keywords

Voluntary wheel running; mouse fitness testing; running endurance; exercise training; exercised-induced health benefits

INTRODUCTION

Voluntary wheel running in the mouse is used as a paradigm to assess physical performance and endurance and also to simulate endurance training in humans. For both, the equipment and basic running procedures are the same, and a description of how to obtain and use running wheels inserted into a standard mouse cage is the subject of this protocol. Wheel running is a voluntary activity and as such is in contrast to other experimental exercise models in mice, which rely on aversive stimuli to force active movement. These include swimming and tread-mill running with an advantage of controlling exercise at reproducible speeds and distances but may not be entirely consistent with normal mouse behavior. Voluntary wheel running on the other hand occurs within routine diurnal rhythmicity patterns in a non-stressed laboratory environment and is less likely to have any confounding effects on molecular mechanisms driving physiological phenotypes induced by the exercise procedure. Mouse strains, gender, age and even individual mice vary greatly in running activity (Coletti et al., 2013; De Bono et al., 2006; Goh et al., 2014), possibly reflecting

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motivation to be physically active in a way that is similar to humans. Whether excessive wheel running by some mice exhibits features of stereotypic behavior, as recently suggested by Richter et al (2014), is not known but might not be different than some humans who obsess with physical activity.

Voluntary wheel running in mice is used for two purposes. The first is as a test for endurance fitness, which is generally less than seven days in duration. The second is as an intervention procedure, since exercise is well recognized as having health-enhancing benefits (Garcia-Valles et al., 2013; Gremeaux et al., 2012). The use of running wheels in experimental mouse models has been greatly enhanced with the availability of slanted wheels that fit into standard sized mouse cages (Figure 1). Each wheel is connected to a computerized activity monitoring system that provides a detailed analysis of voluntary wheel running for individual mice.

BASIC PROTOCOL

The basic protocol consists of allowing mice to run freely on the open surface of a slanted plastic saucer-shaped wheel placed inside the mouse cage. Mice run during the active dark cycle and rarely run during the inactive light cycle. Rotations are electronically transmitted to a USB hub so that frequency and rate of running can be captured. Mice are individually housed so that accurate recordings can be made for each animal.

When mice are tested for endurance fitness, the test time depends on the background strain and age, but should be less than seven days to help prevent the possibility of triggering a long term physiologic response that may affect the mouse phenotype. For example, six days was adequate to show a difference in Klotho-deficient mice on a C57BL/6 background (Phelps et al., 2013). In another study conducted in the Ladiges lab, a three-day fitness test was able to detect differences in running distances between young and old mice (Figure 2). Before the running assessment starts, wheels are locked for 48 hours in order to acclimate mice to the wheel and reduce any novel object behavioral disturbances. When voluntary wheel running is used as a means of endurance training to determine health benefits, the setup procedures are the same but the duration is longer depending on the nature of the experiment. Some studies may call for pre-training periods before any experimental conditions are imposed on the animals. An example would be a running period before transplanting tumor cells, which can have an anti-tumor affect as we recently reported (Goh et al., 2014)

Equipment and Materials

1. **Standard cage with food hopper and bedding**

2. **Running wheel**

   The free-wheel running apparatus by Med Associates Inc. (St Albans, VT, USA) is an exercise monitoring system that consists of a plastic slatted wheel slanted on a central rod planted into a support base that houses the batteries. The entire unit fits into most standard mouse cages including those designed for controlled air flow (Figure 1). Rotation of the wheel by the mouse transmits an electronic signal wirelessly to a hub and the number of revolutions is recorded on the Wheel Manager software (Med Associates Inc) every minute. Activity is recorded as the distance covered across time, and the data is exported to a spreadsheet (e.g., Excel).

Other systems, such as the Mouse Home Cage Running Wheel (Columbus Instruments, Columbus, OH) are available that provide a similar means for voluntary wheel running.

2. **Locking pins (supplied with running wheel)**

   Locking pins are needed to lock running wheels for the non-running control cohort.

3. **Magnet. (supplied with running wheel)**

   A magnet is needed to reset the revolutions on each running wheel.

4. **Wireless environmental sensor**

   The wireless environmental sensor (ENV-044-01) sends wheel rotation signals from each wheel to the monitoring hub.

5. **Triple A (AAA) batteries**

   Three AAA batteries are needed for each wheel unit to operate electronically.

6. **Monitoring hub**

   The monitoring hub (DIG-804, USB interface hub) receives a signal for the number of wheel rotations every 60 seconds from the environmental sensor,, and can manage up to 40 wheels. Messages are relayed to the wheel manager software for data storage and retrieval.

7. **Software**

   The software program consists of the Wheel Manager Software (SOF-860), which collects and stores data from the wheel rotation sensor as relayed by the USB interface hub, which manage up to four interface hubs concurrently. The Wheel Analysis Software (SOF-861) is additional software that generates actograms and periodograms.

8. **Computer**

   A standard lap top computer is sufficient.

Protocol Steps

1. **Determine the number of wheels needed.**

   Mice will be housed individually so a separate cage is required for each wheel. The number of cages will be determined by the number of mice in each cohort. Depending on the experimental design, the non-running cohort usually is placed with running wheels that are locked so that there is exposure to the running wheel but the mouse is not able to rotate it. This controls for movement associated with getting on and off the wheel.

2. **Load the software into a standard computer dedicated to running wheels.**

   The software program can handle up to four hubs. Each hub can accommodate up to forty individual running wheels. The software is a commercial program specifically for Med Associates running wheels.

3. **Place the wheels in individual standard mouse cages.**

   If the wheels are placed toward the back of the cage, the automatic water lixit may be covered. If this is the case, standard water bottles must be used (Figure 1). The standard wire top lid with a food delivery drop-down bin may be replaced with a flat lid to make more room for the mouse while running, so a food container must be placed on the floor of the cage. A heavy glass cup is useful for this purpose (Figure 3).

4. **Verify of the wireless connection**

   It is essential to verify that the wireless connections are intact and signals can be made between the running wheel and the interface hub, and from the interface hub to the computer program before starting the experiment. Each hub will have a reference channel number that needs to be matched to the channel numbers on the wheels. For instance, if the monitoring hub uses channels 1, 2, and 3 (in the down position), then the 40 running wheels would need to have the same channels (but flipped in the up position). A different combination of channels will be needed for the additional wheels. Once the channels have been set, the wheels' battery packs are disconnected, and the power is turned off for the monitoring hub and the computer. After three minutes, the computer is powered up, followed by the monitoring hub. The Wheel Manager program is started by choosing "Tools" and "Delete All Wheels". The wheel battery packs are connected in the order that they should appear (e.g. 001, 002). Once all of the wheel battery packs have been connected, they are ready for experimentation. The wheels are tested by rotating them slowly, which allows the rotations to be picked up on the Wheel Manager program thus verifying the wireless connection. The magnet provided by the manufacturer is then placed over the axle of the plastic base to reset the number of revolutions to zero on the Wheel Manager program.

   Because the running wheel system is based on WIFI signaling, no cell phones, WIFI routers or other electronic devices with WIFI connectivity should be allowed in the same room when the study is being conducted.

5. **Transfer mice to individual running wheel cages.**

   Generally this involves transferring mice that are group housed to a single housing cage with a running wheel. In order for each mouse to become accustomed to the strange object (the running wheel) and also the individual housing environment, a 48 hour acclimation period is recommended with wheels in the locked position using the locking pins provided by the manufacturer.

6. **Unlock the wheels to begin the experiment.**

   The wheels are unlocked for the experimental cohort so the experiment can start, but the wheels for the control cohort (non-running) remain locked. Mice in the experimental cohort are allowed to freely use the wheels during both dark active and light inactive cycles so use of the wheel can be monitored on a 24 hour basis. Rotation recordings are made every 60 seconds and integrated into the computer program.

7. **Monitor the collection of data on a regular basis.**

   Data collection can be obtained at a resolution of one-minute intervals, with the intervals easily customizable by the user. Because the running wheels operate on three AAA batteries, it is essential that the voltage be checked on a regular basis on the Wheel Manager interface to ensure that data will not be lost due to loss of power. Generally, the batteries are sufficient for a period of at least one month of daily wheel running. It is also recommended that data be downloaded a few times a week to ensure collection and storage are not affected in the event of a building-wide power loss.

8. **Determine the duration of running by the experimental objective**

   The use of voluntary wheel running to assess fitness is a frequently used test in mice, especially in aging studies. The duration depends on the objective of the experiment but is generally limited to three to six days. The experiment is generally terminated by removing the mice and returning them to group housing. The use of voluntary wheel running for endurance training is usually as an intervention for a health related problem or condition, which by definition is a long term experiment. For example, if the study involves determining the effect of exercise training on lifespan, the experiment is terminated at the end of life for each mouse. For tumor experiments, the study may continue until tumor burden reaches a particular endpoint for the non-running mice.

COMMENTARY

Background

The use of voluntary wheel running in mouse physiology studies was enhanced by the description of slanted wheels instrumented with a computerized monitoring system (De Bono, 2006). The unit was designed to fit in a standard mouse cage, allowing experiments to be conducted without specialized equipment or caging. Other running wheel designs, e.g., the upright circular design described by Richter et al. (2014), have been used.

Critical parameters and troubleshooting

While the voluntary wheel running paradigm has a number of strengths as an exercise training model, some have questioned whether the intensity and duration of wheel running done by the mice compares to a reasonable exercise program for people. It is generally accepted that most all mice, regardless of strain or age, will run voluntarily, with older mice running less compared with younger mice. Running distance and rate are strain dependent, with the common BALB/c and C57BL/6 considered average to above average distance runners (DeBono et al., 2006; and unpublished observations). In a recently published breast cancer study, old BALB/c females that ran an average daily distance of close to 5 km during the 12 hour active night cycle period had less tumor burden than mice that ran an average daily distance of less than 2 km per night (Goh et al., 2014). The 5 km per night is less than one half a km per hour, which is very attainable by many older women in various types of exercise training settings. The question is whether one hour of training would provide the same protective anti-tumor benefits as 12 hours of voluntary wheel running. Mice run in spurts with periods of intense running and periods of light running or no running, as shown in the actogram in Figure 4. Mice run most intensely the first several hours of the beginning of the active dark cycle, and it has been shown that one hour of running five days a week generates physiological responses (Goh et al., 2013). Therefore, more studies are needed to assess the health benefit of a one-hour per day running time in mice with measurable disease phenotypes.

Another question is whether energy expenditure needs to be comparable. If the average stride of a female mouse is 5 cm and the average stride of an older woman is 135 cm, then a comparable one hour running distance would be about 10 km. A 10 km run over one hour is probably doable by many older women but most likely not a reasonable expectation. However, if the same energy expenditure needed to complete the 10 km run were compressed into a shorter time frame with more enticing but comparable energy demanding types of exercise training, then older women might be more enthused about participating. The metabolic parameters of energy expenditure can easily be measured in mice engaged in exercise training experiments. These same parameters can also be easily measured in women so that relative energy expenditures can be compared to help further validate voluntary wheel running as a translational model for exercise training.

The patterns of voluntary wheel running vary between mouse strains, gender, age, and individual mice (Figure 2). This variation must be accounted for when designing studies. The variation between individual mice may create wide standard deviations so that averaging may not be possible without using large numbers of mice. However, the individual differences can be captured as increasing or decreasing curves and correlated with other parameters. For example, it has been shown that mice running longer distances have decreased breast cancer progression (Goh et al., 2014).

Because some strains of mice such as C57BL/6 show fluctuating running distance patterns over time (Figure 5), it is advisable to account for this in experimental design. For example, an average daily running distance over two weeks may be different than if averaged over an entire month.

Anticipated Results

The fitness testing paradigm for voluntary wheel running is straightforward and has been used to measure endurance in mice with compromised skeletal muscle function. C57BL/6 female mice deficient in the klotho protein subjected to voluntary wheel running for six days showed a sporadic running pattern characteristic of repeated bouts of exhaustion (Phelps et al., 2013). The mice ran at the same speed as wild type controls but spent significantly less time running. Therefore, voluntary wheel running served as a measure of muscle performance. Voluntary wheel running can also detect muscle performance differences in young and old mice. The Ladiges lab showed that 4, 12, 20 and 28 month old C57BL/6 and CB6F1 male mice subjected to three days of wheel running ran decreasing distances with increasing age (Figure 2). Pearson's correlation analysis was used to assess relationships between distance ran by runners and dependent variables such as tumor burden

Time considerations

Fitness assessment using voluntary wheel running can be completed in as little as five days, but the duration is determined by the experimental objectives. The use of voluntary wheel running for endurance training is a long term study. For example, if the study involves determining the effect of exercise training on lifespan, the experiment is terminated at the end of life for each mouse. For tumor experiments, the study may continue until tumor burden reaches a particular endpoint for the non-running mice.

Acknowledgments

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LITERATURE CITED

Coletti D, Berardi E, Aulino P, Rossi E, Moresi V, Li Z, Adamo S. Substrains of inbred mice differ in their physical activity as a behavior. Scientific World Journal. 2013; 2013:237260. Epub 2013 Mar 6. 10.1155/2013/237260 [PubMed: 23533342]

De Bono JP, Adlam D, Paterson DJ, Channon KM. Novel quantitative phenotypes of exercise training in mouse models. Am J Physiol Regul Integr Comp Physiol. 2006 Apr; 290(4):R926–34. Epub 2005 Dec 8. [PubMed: 16339385]

Garcia-Valles R, Gomez-Cabrera MC, Rodriguez-Mañas L, Garcia-Garcia FJ, Diaz A, Noguera I, Olaso-Gonzalez G, Viña J. Life-long spontaneous exercise does not prolong lifespan but improves health span in mice. Longev Healthspan. 2013 Sep 16.2(1):14.10.1186/2046-2395-2-14 [PubMed: 24472376]

Goh J, Endicott E, Ladiges WC. Pre-tumor exercise decreases breast cancer in old mice in a distance-dependent manner. Am J Cancer Res. 2014 Jul 16; 4(4):378–84. eCollection 2014. [PubMed: 25057440]

Goh J, Ladiges WC. A novel long term short interval physical activity regime improves body composition in mice. BMC Res Notes. 2013 Feb 19.6:66.10.1186/1756-0500-6-66 [PubMed: 23422015]

Gremeaux V, Gayda M, Lepers R, Sosner P, Juneau M, Nigam A. Exercise and longevity. Maturitas. 2012 Dec; 73(4):312–7. Epub 2012 Oct 11. 10.1016/j.maturitas.2012.09.012 [PubMed: 23063021]

Phelps M, Pettan-Brewer C, Ladiges W, Yablonka-Reuveni Z. Decline in muscle strength and running endurance in klotho deficient C57BL/6 mice. Biogerontology. 2013 Dec; 14(6):729–39. Epub 2013 Sep 13. 10.1007/s10522-013-9447-2 [PubMed: 24030242]

Richter SH, Gass P, Fuss J. Resting Is Rusting: A Critical View on Rodent Wheel-Running Behavior. Neuroscientist. 2014 Jan 6; 20(4):313–325. [PubMed: 24395338]

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Figure 1.

The free-wheel running apparatus (Med Associates Inc, St Albans, VT, USA) fits into a standard size mouse cage. Other systems, such as the Mouse Home Cage Running Wheel (Columbus Instruments, Columbus, OH) are available that provide a similar means for voluntary wheel running.

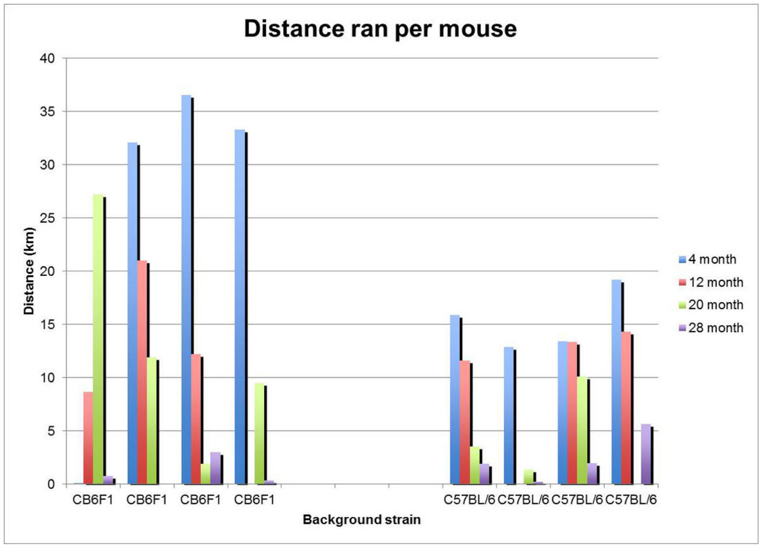

Figure 2.

Wheel running distance varied in male mice by strain, age, and individual mouse. Mice were either CB6F1 or C57BL/6. Each bar represents one mouse. All mice ran for a three-day period.

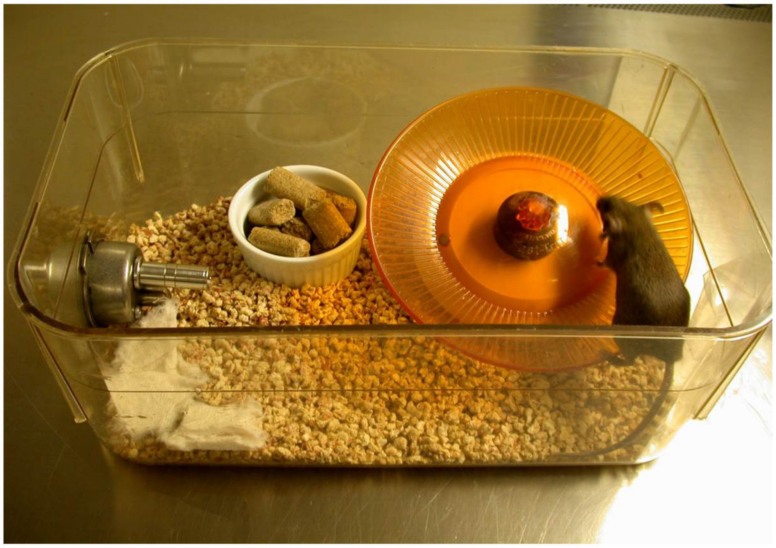

Figure 3.

The running wheel unit is placed in a cage where the hanging food hopper has been replaced with a thick glass cup placed on the cage floor as a food holder.

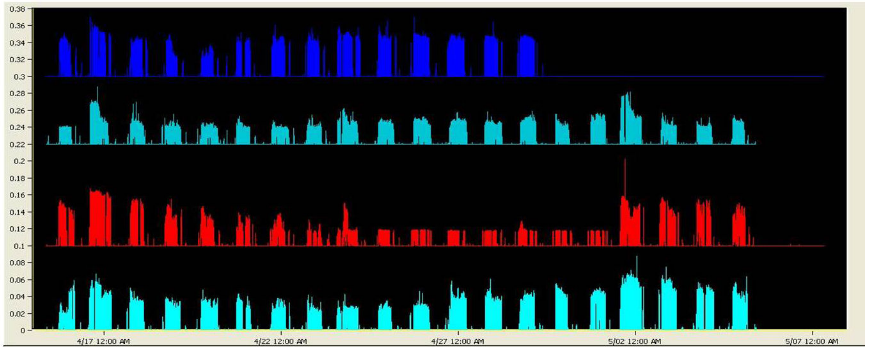

Figure 4.

The running frequency actogram shows that four mice differed in the amount of time and distance on the running wheel. Running occurred almost exclusively during the active dark cycle.

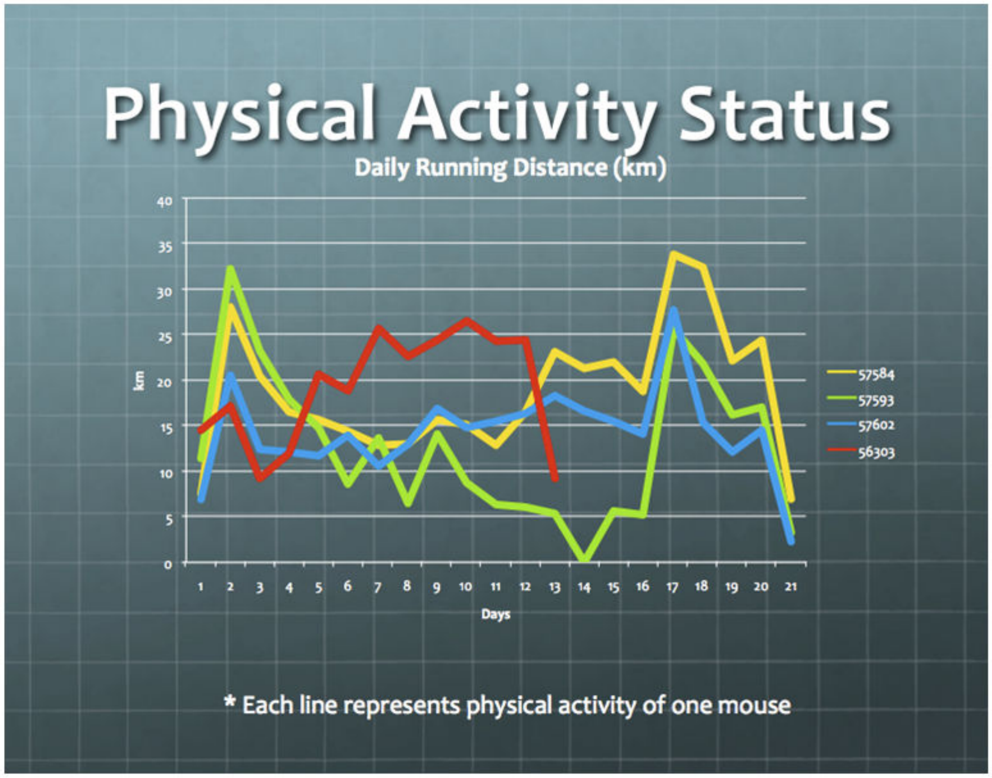

Figure 5.

Five-month-old C57BL/6 male mice had a fluctuating running distance pattern over a 21 day period with an initial burst of activity up to day 3 or 4, followed by several bouts of decreasing and increasing activity over the remaining time period. Each line represents the activity of one mouse.