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Original research article

Using wheel availability to shape running behavior of the rat towards improved behavioral and neurobiological outcomes

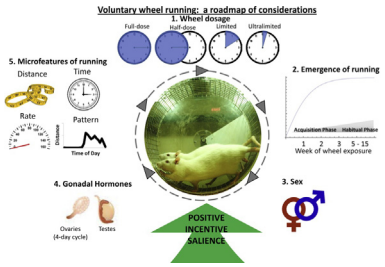
Julia C. Basso*, Joan I. Morrell

Rutgers University, Center for Molecular and Behavioral Neuroscience, 197 University Avenue, Newark, NJ 07102, USA

HIGHLIGHTS

- These experiments show how to shape voluntary wheel running (VWR) behavior.
- Variables manipulated include wheel access, sex, hormones, and wheel apparatus.
- We hypothesize that VWR can be shaped because of its positive incentive salience.
- Results show how to optimize the effect of VWR on both behavior and neurobiology.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Though voluntary wheel running (VWR) has been used extensively to induce changes in both behavior and biology, little attention has been given to the way in which different variables influence VWR. This lack of understanding has led to an inability to utilize this behavior to its full potential, possibly blunting its effects on the endpoints of interest.
New method: We tested how running experience, sex, gonadal hormones, and wheel apparatus influence VWR in a range of wheel access "doses".
Results: VWR increases over several weeks, with females eventually running 1.5 times farther and faster than males. Limiting wheel access can be used as a tool to motivate subjects to run but restricts maximal running speeds attained by the rodents. Additionally, circulating gonadal hormones regulate wheel running behavior, but are not the sole basis of sex differences in running.
Comparison with Existing Method(s): Limitations from previous studies include the predominate use of males, emphasis on distance run, variable amounts of wheel availability, variable light-dark cycles, and possible food and/or water deprivation. We designed a comprehensive set of experiments to address these inconsistencies, providing data regarding the "microfeatures" of running, including distance run, time spent running, running rate, bouting behavior, and daily running patterns.
Conclusions: By systematically altering wheel access, VWR behavior can be finely tuned – a feature that we hypothesize is due to its positive incentive salience. We demonstrate how to maximize VWR, which will allow investigators to optimize exercise-induced changes in their behavioral and/or biological endpoints of interest.

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1. Introduction

Wheel running in rodents is widely used as a behavioral tool to induce changes in a variety of important dependent variables

* Corresponding author. Present address: New York University, Center for Neural Science, 4 Washington Place, Room 809, New York, NY 10003, USA.
E-mail addresses: jb4590@nyu.edu, juliacolette@gmail.com (J.C. Basso), jmorrell@andromeda.rutgers.edu (J.I. Morrell).
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in the central nervous system. These variables include increases in neurogenesis, gliogenesis, and vasculogenesis/angiogenesis, as well as enhancements in synaptic plasticity (Vaynman and Gomez-Pinilla, 2005; Vivar et al., 2013; Voss et al., 2013). Wheel running also results in improvements in behavioral measures of learning, memory, attention, anxiety, depression, and addiction (Cotman and Engesser-Cesar, 2002; Voss et al., 2013). Studies that have employed wheel running as a behavioral and biological catalyst are likely to be influenced by the amount and nature of wheel running behavior. Therefore, giving substantial consideration to the way in which rodents voluntarily engage with the wheel and determining how this behavior can be manipulated is an important area of inquiry.

Since Stewart first used the wheel in 1898 to investigate the effects of alcohol, barometric pressure, and diet on daily activity (Stewart, 1898), studies on wheel running in rodents are abundant, including over 3000 citations in PubMed. Collectively these studies demonstrate that wheel running behavior varies greatly depending upon the subject's age, body weight, and endocrine status (Afonso and Eikelboom, 2003; Meijer and Robbers, 2014; Sherwin, 1998). In addition, wheel availability and wheel shape and size as well as the presence of a sexual partner, pregnancy, food availability, and changes in the light-dark cycle can also affect wheel running patterns (Sherwin, 1998).

For the investigator seeking to deliberately design a wheel running intervention, the parametric comparability and detail of this literature are limited by several factors. These include the predominant use of male subjects, an emphasis on distance run (as opposed to time spent running, running rate, running patterns, *etc.*), differences in wheel apparatuses, variable amounts of wheel availability (with unacknowledged impact of wheel deprivation in between periods of running), variable light-dark cycles, and inclusion of food and/or water deprivation. Furthermore, studies conducted before more advanced standards of husbandry, housing, or running wheel conditions may have introduced unrecognized confounding stressors, yielding non-normative data outcomes.

Here, we report a comprehensive set of experiments that are designed to address some of the above limitations and inconsistencies in the literature. We did this by testing how variables such as running experience, sex, gonadal hormonal status, and wheel apparatus influence both the emergence of stabilized running and the expression of habitual running behavior when wheel availability is provided across a range of "doses" from *ad libitum* to ultralimited (*i.e.*, only 30 min daily). These experiments provide data beyond total distance run to include variables such as the time spent running, running rate, bouting patterns (*i.e.*, the way in which rodents run for minutes at a time), and details regarding the daily cycle of running. The consideration of these factors allowed for a detailed analysis of quantitative comparability across these variables.

Our experiments were designed to incorporate methodological features based on two key prevailing interpretations in the literature. First, these experiments use only voluntary wheel running, as it is associated with a myriad of positive effects in rodents, while forced exercise is a known stressor in rodents (Brown et al., 2007; Moraska et al., 2000). Second, the experiments were designed on the premise that voluntary wheel running has positive incentive salience for rodents. This interpretation is based on their robust unconditioned and conditioned responses to wheel availability, and data demonstrating that such responses are regulated by brain regions that are part of a neural network necessary for most motivated behaviors (Basso and Morrell, 2015; Belke, 1997; Belke and Wagner, 2005; Collier and Hirsch, 1971; Greenwood et al., 2011; Iversen, 1993; Kagan and Berkun, 1954; Premack et al., 1964; Sherwin, 1998). As we recently identified, the positive incentive salience of wheel running applies to both the emergence of wheel running in wheel naïve subjects (termed the acquisition phase) and its stabilized, habitual phase (Basso and Morrell, 2015). Thus, by varying the availability ("dose") of the wheel, including *ad libitum* (every-day access), half-dose (alternate-day access), limited (hours per day), and ultralimited (minutes per day) wheel access periods, we were able to modulate wheel running behavior similar to the process of altering the dose of stimuli with positive incentive salience, such as any natural or pharmacological reward. As we intended to have our main variable be a running treatment, we deliberately avoided changing the circadian rhythm of the rats' natural running patterns, which is to conduct virtually all running during the dark period. The impact of this systematic variation in wheel access was intended to allow investigators to use wheel running as a tool to induce specific responses tailored to their biological and behavioral end points.

2. Methods

2.1. Subjects

Data were collected from both post-weaning (PND21) as well as adult (PND65 and older) male and female CD/Sprague Dawley rats. The original stock of rats came from Charles River Laboratories (Kingston, NY, USA), and all animals utilized in these experiments were bred in our colony according to an IRB-approved protocol at the Rutgers University Laboratory Animal Facility (Newark, NJ, USA; accredited by the American Association for Accreditation of Laboratory Animal Care). Stud males were purchased twice a year to ensure efficient stud service and to avoid inbreeding or genetic drift of the Newark Colony from the Charles River source; virgin females were added as needed for the same reason. Breeding to provide the experimental subjects was carried out by a timed mating protocol that resulted in offspring 22 days after mating. Offspring were weaned between PND 21 and 28, and then subjects were housed in groups of two to four until they reached the age needed for the particular experiments. All animals were kept on a 12-h light-dark cycle (lights on at 7:00 am; unless otherwise noted) in a room maintained at 22(±1)°C and given *ad libitum* access to water and rat chow (Lab Diet 5008, PMI Nutrition International, LLC, Brentwood, MO, USA). Daily checks were conducted for health and availability of food and water. Twice per week, animal weights were recorded and animal husbandry was performed. All animals were healthy and had normal body weight throughout all experiments. Animal care and experimental procedures performed in this protocol were in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, revised 1996) and were reviewed and approved by the Rutgers University Animal Care and Facilities Committee. Care was taken to minimize animal suffering as well as the total number of animals utilized.

2.2. Running wheel apparatuses

Animals were housed in either an AccuScan Instruments (Columbus, OH, USA) VersaMax Animal Activity Monitor (wheel: 25 cm diameter, 9.45 cm width, stainless steel mesh floor (grid boxes 1.2 cm sq); home cage: 40 cm long × 40 cm wide × 30 cm high) or a Med Associates Inc. (St. Albans, VT, USA) ENV-046 Activity Wheel with Plastic Home Cage for Rat (wheel: 35.6 cm diameter, 9.69 cm width, 4.8 mm stainless steel grid rods with 1.6 cm spacing, 12 g freewheeling drag; home cage: 48.26 cm long × 26.67 wide cm × 20.32 cm high with a 7.2 cm wide × 10.2 cm high opening to wheel). The resistance of both running wheels was low and nearly equivalent, and no extra weight or resistance was placed on either wheel. Both housing apparatuses were solid floors lined with woodchip bedding (Beta chip, Northeaster Prod-

ucts Corp., Warrensburg, NY, USA). In the AccuScan system, 16 infrared beams lined each axis of the box, which enabled measurement of wheel running activity. Data were captured electronically using Windows-based software, VersaMax, VersaDat or MedPCIV. All data were captured in the AccuScan system except for those experiments conducted to assess differences between the two apparatuses. The accuracy of the computer-recorded wheel turns was confirmed at the start and finish of each experiment using additional manual counters. Animals were periodically monitored to ensure that wheel turns were from running rather than from the wheel continuing to turn once the rat stepped off the wheel or from the rat interacting with the wheel in ways other than running.

2.3. Procedures

2.3.1. Full-dose methods: maximum wheel access

At age 65 days, males (n = 6) and females (n = 6) previously housed in shoebox cages and naïve to running wheels were placed in the AccuScan Instruments system boxes with running wheels at ~12:00 p.m. (lights on at 7:00 a.m., off 7:00 p.m.) which served as their home cages, providing *ad libitum*, that is continuous, access to the wheels for 1–15 weeks, as well as *ad libitum* access to food, water, and additional cage space for exploration and sleeping. An additional group of males (n = 4) and females (n = 8) were placed in the running wheel apparatus immediately after weaning (PND 21), and their running was followed for the next 15 weeks.

2.3.2. Limited wheel access

Females were used for all limited wheel access and apparatus comparison experiments because they are more robust runners than males (see results Section 3.1). Intact females also gained less weight over the lengthy experiments, an important variable as during 5–15 weeks of wheel exposure, males gained considerable weight, and those over 500 g rarely ran more than 1 km (Section 3.1 Fig. 1, bar 4). Females also provide novel data, as most voluntary wheel running studies have been conducted using males (Eikelboom and Lattanzio, 2003; Lattanzio and Eikelboom, 2003). For these studies, females were provided with the wheel apparatus as of PND 65, that is, as young adults.

2.3.2.1. Using alternate-day wheel access to achieve "half-dose" running. Subjects (n = 20) were given access to the AccuScan system with running wheels for 24 h every other day for a total of 21 days of running (on non-running days, animals were housed in shoebox cages). Access was given specifically during the light cycle (~1:00 pm) so that a change in running could be observed in situations of a rebound running response (rats with *ad libitum* access did not run or ran minimally [<0.1 km] during this phase of the light cycle). In addition, access was provided for a full 24 h to avoid the complexity of forcing the rats to change their significant preference for carrying out the majority of their running in the dark.

2.3.2.2. Workout doses; limited or ultralimited running wheel access. Subjects were randomly assigned to two groups, 30-min (ultralimited; n = 8) or 2-h runners (limited; n = 8), and when not with their wheel, housed in pairs in shoebox cages. Each day for 38 days, 2 h after lights off (*i.e.*, the normal active period), animals were individually placed in their wheel apparatus and allowed to run for 30 min or 2 h.

2.3.3. To examine the impact of gonadectomy on running

Male (n = 5) and female (n = 5) rats experienced in running (21–38 days of wheel access) underwent gonadectomy or sham gonadectomy. All animals were first anesthetized with a standard mixture of ketamine, xylazine, and acepromazine maleate. Surgical procedures involved opening the body wall or scrotal sac, removing or handling gonads with surgical instruments, and closing the area using wound clips. These methods were in accordance with standard procedures and were conducted using aseptic conditions (Waynforth, 1983). To allow wound healing and endogenous levels of gonadal hormones to fall to undetectable levels, 1 month elapsed before animals were allowed *ad libitum* access to running wheels for 3 weeks.

2.3.4. To examine the effect of estrus cycle stage on running

These studies were conducted in females (n = 8) that had at least three weeks of running experience in the Med Associates wheel systems under full-dose or *ad libitum* wheel access conditions. To determine the stage of their cycle, vaginal lavage was performed by standard procedure (Waynforth, 1983) uniformly each day during the middle of the light cycle (12:00–1:00 pm) (*i.e.*, the normal rest period). The slides were examined by one observer and confirmed by a second observer both using a Zeiss bright-field microscope. Cell cycle stages were determined by comparison with photomicrographs in *The Laboratory Rat* (Sharp et al., 1998) and *The Laboratory Rat: Volume I Biology and Diseases* (Baker et al., 1979). Samples were analyzed randomly, and both observers were unaware of experimental conditions. Because of the dynamics of the continuous estrus cycle (Feder, 1981), each sample is considered in the context of the sequential days of sampling, and therefore represents not a single absolute stage of the estrus cycle but a process of transition from metestrus-diestrus, diestrus-proestrus, proestrus-estrus, and estrus-metestrus, labeled in Fig. 3 as metestrus, diestrus, proestrus and estrus, respectively.

2.4. Analytic approaches and statistical analysis

No experimental subjects were excluded, as all engaged with the running wheels offered. All wheel running data were analyzed by examining wheel turns captured in one minute bins throughout each day that the animal had access to the wheel. We observed that few wheel turns were made by the rat interacting with the wheel from the inside or outside rather than running in it; these distances were not significantly different from zero. Daily distance run was calculated by summing total wheel turns in each 24-h session. Wheel turns were then converted into distance (kilometers) by multiplying this number by the circumference of the wheel. Daily time in the wheel was calculated by counting the number of minutes that the rat ran at least 1 wheel turn in each 24-h period. Daily rate of running was calculated by dividing daily distance run by the daily time spent running in the wheel. For all analyses, we defined a running bout as a sequence of time bins (1 min each) that had at least one recorded wheel turn. The duration of these bouts and the distance run during each bout were calculated as above. The rate of the bouts, termed *bout rate*, was calculated by dividing the bout distance by the bout time. Although bout rates are similar to the hourly or daily rates, due to the way these values were calculated, they are not intended to be exactly the same. Unless the term *bout rate* is used, we are referring to an hourly or daily rate. We acknowledge that automated wheel running systems such as the ones used here may not accurately assess instantaneous running speeds, thus giving imprecise measurements of the duration and frequency of running bouts (Eikelboom, 2001; Girard et al., 2001); however, the data presented here are calculated at the utmost precision possible within the limitations of the current wheel running hardware and software. Finally, data for weekly wheel running distances are reported as averages of data captured on each day of the week from each subject.

All statistical analyses were conducted using IBM® SPSS® 16.0 graduate pack, 17.0, or 19.0 (Chicago, IL, USA), or GraphPad Prism® 5.0 (LaJolla, CA, USA) (used for linear regression analyses only).

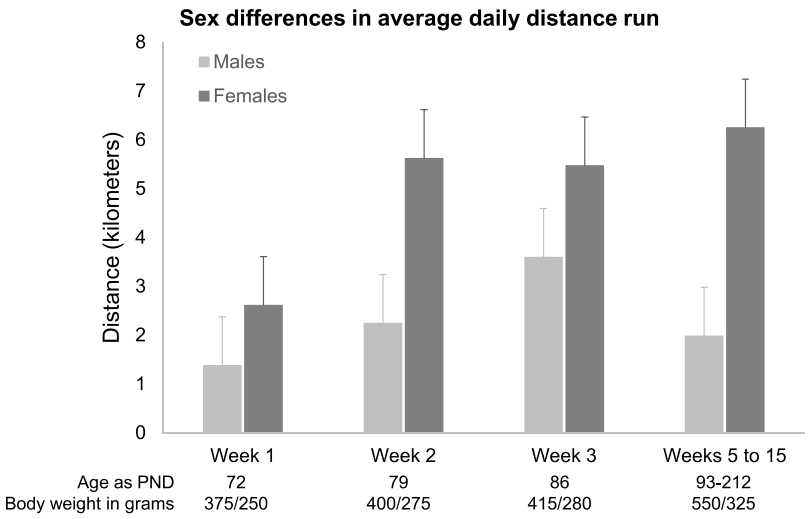

Fig. 1. Data are presented as average (±SEM). The impact of full-dose wheel access on distance traveled during the transition from naïve to habitual wheel running. Wheel running emerged within the first three weeks of wheel availability, with females reaching peak distances by week two and males by week three. Once habitual running distances were reached, females ran on average 1.5 times farther than males.

A significance value of $p \leq 0.05$ was used for all statistical analyses; data met the tests of normalcy and homogeneity of variance (assessed *via* Levene's test) and so were analyzed with parametric tests. If data did not meet sphericity, then a greenhouse-geisser correction was used. *Numerical data*: An independent samples *t*-test was used to determine all statistical significance between one measure in two separate groups. A repeated-measures analysis of variance was used to determine statistical significance between measures sampled in the same set of animals on two or more occasions. Linear regression was used to determine statistical significance between independent groups of animals where data were repeatedly sampled over an extended period, for example, on 21 or more days, and it was appropriate to fit a trend line to the data. A Kolmogorov-Smirnov two-sample test (K-S test) was conducted when statistically significant differences needed to be determined between two frequency distributions.

3. Results

3.1. Characteristics of running with maximum (ad libitum) wheel access; the full-dose running treatment

3.1.1. Daily distance run

A gradual and steady increase in average daily distance run occurred from the first day of wheel exposure in wheel-naïve rats for both males ($F(1.250,33.757) = 27.939$, $p < 0.001$) and females ($F(1.338,36.116) = 10.605$, $p = 0.001$), stabilizing into peak habitual running after 2 weeks for females and 3 weeks for males (Fig. 1, first three columns). During the emergence of stable running, no correlation between body weight and the distance run were seen in either sex ($p > 0.05$; all weights were taken at the end of the indicated running week, whereas weights for weeks 5–15 were an average of data points taken during that time period). At the end of this emerging running period (week 3), females ran on average 1.5 times farther daily than males (males 3.6 kilometers [2.2 miles]; females 5.5 kilometers [3.4 miles] per day).

Once stabilized running was achieved, examination of the subsequent 5–15 week period demonstrated that while running distances in cycling females remained at, or slightly above, the levels found at the three week mark, running began a gradual decline in males with steady increasing body weights ($t(15.816) = 2.395$, $p = 0.029$; Fig. 1 last column). Specifically, intact males over 500 g rarely ran over 1.0 km per day. Over that same time period, intact females remained at stable body weights and distances run, including the normal variation across the daily cycle, such that after 3 weeks and up to 15 weeks, no decline in their running was seen ($t(5.615) = -1.741$, $p = 0.136$).

A thorough, quantitative analysis showed that if rats were exposed to the running wheel as soon as they were able to engage in spontaneous, well-organized running behavior, which in our hands was PND 21, this did not impact the basic pattern and distance of stabilized running compared to those described in the two paragraphs above, which were first offered the wheel as young adults (PND 65). A group of females, initially exposed to the running wheel on PND 21 (pre-pubertal juveniles), were running a stable 6 kilometers per day by the end of week three as *peri*-pubertal adolescents (PND 42). These distances were similar ($p > 0.05$) to those run by females that were first exposed to the running wheel on PND 65 (young adult), running an average daily stable maximum of 5.5 kilometers per day as cycling adults (PND 86).

3.1.2. Time and time of day spent running

At 3 weeks of wheel exposure, males and females spent equal time running on the wheel per day (males 4.3 h, females 3.7 h, calculated as a weekly average). As rodents are nocturnal, 90% of running was conducted during the dark phase of the light-dark cycle. Both sexes conducted significantly more running, up to 60% of their total, in the first 6 h of the dark period and then significantly less in the last 6 h (males $t(27) = 6.267$, $p < 0.001$; females $t(27) = 4.151$, $p < 0.001$). The only significant sex difference related to time was that once stabilized running patterns were established (*i.e.*, by week 3), females ran for 25% more time in the second six hours of the dark period than males ($t(54) = -2.257$, $p = 0.028$).

3.1.3. Rate of running

Both males ($F(2,50) = 33.149$, $p < 0.001$) and females ($F(1.336, 36.075) = 23.424$, $p < 0.001$) increased their average daily running rate during the first 3 weeks of wheel exposure, with females peaking at week 2 and males at week 3. Females ran at significantly faster average rates than males during the first three weeks of wheel exposure (week 1 $t(35.608) = -5.001$, $p < 0.001$; week 2 $t(37.450) = -7.513$, $p < 0.001$; week 3 $t(54) = -4.066$, $p < 0.001$). Thereafter, females ran on average 1.5 times faster (19.2 m/min [0.7 mi/hr]) than males (13.5 m/min [0.5 mi/hr]). Additionally, the average fastest rate run by females (50.0 m/min), that is, the average fastest rate achieved by each individual during days 14–21 of

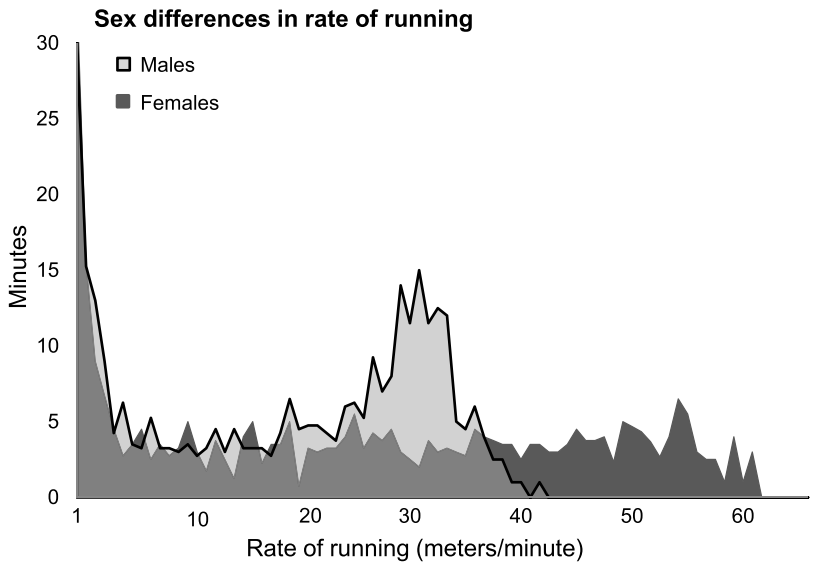

Fig. 2. The impact of full-dose wheel access on running rate in habitual runners (week 3). Females spend more time running at faster rates whereas males spend more time running at moderate rates. Overall, females run on average 1.5 times faster than males.

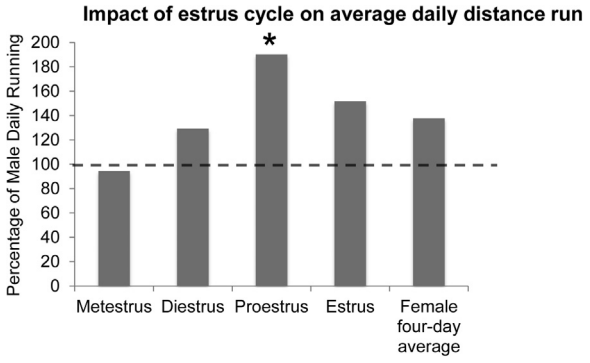

Fig. 3. The impact of gonadal hormones on daily distance run. In this graph, daily running by females across the estrus cycle is expressed as the percentage of male daily running, which is set at 100% and represented by the dashed line at 100% in the graph. Compared to other periods of the estrus cycle, females run the greatest distances during the proestrus period. Daily distances run during other periods of the estrus cycle are greater than those run by males, but do not reach statistical significance.

wheel exposure, was 1.5 times greater than the average fastest rate run by males (32.7 m/min) ($t(43.931) = 10.720$, $p < 0.001$).

A frequency distribution histogram of the number of minutes spent at various rates of running (meters per minute) demonstrated that overall, females spent more minutes running at higher rates and males spent more minutes running at lower rates (Fig. 2) (K-S tst, $p < 0.05$). The similar initial modes (0.8 m/min) of both sexes was due to the number of minutes spent running 0.1–4 m/min and were observed to be mostly due to walking or slow running in the wheel.

3.1.4. Microfeatures of running patterns

Subjects ran in short periods, referred to as running bouts, separated by periods in which they rested, ate, drank, groomed, or otherwise locomoted. Sex differences were not a prominent feature of bouts, and so the data represented in Table 1 (column one) are pooled male and female data. The pattern of running in bouts, interspersed with other activity, occurred at a similar frequency of 4 bouts per hour averaged overall for the dark period. The average length of a bout, the time in between bouts, and the distance traveled during bouts remained consistent during the 1st and 6th hour of the dark cycle, but significantly decreased by the 12th hour (6th *versus* 12th hour: bout time $t(32) = 4.060$, $p < 0.001$; bout distance $t(32) = 4.416$, $p < 0.001$; bout rate $t(31) = 6.195$, $p < 0.001$). The longest bouts were in the first hour of darkness, when subjects covered about 110 m per bout in five minute bouts. The shortest bouts were in the last hour of darkness when subjects ran for only 2 min at a time, covering approximately 9 m.

3.2. Influence of gonadal hormones

3.2.1. Ovarian cycle hormones

In habitual runners, gonadal hormones were significant drivers of sex differences in running distances (Fig. 3). Metestrus females ran similar distances to males, while diestrus and estrus females ran somewhat, but not significantly, more than males or metestrus females. The significant differences in running appeared in the proestrus female, which spent almost double the time (6.05 h) running, and ran 3 times longer distances (9.4 km) at 54% faster rates (25 m/min), in longer bouts, than their metestrus (or male) counterparts (time $t(10) = 7.196$, $p < 0.001$; distance $t(6.014) = 4.293$, $p = 0.005$; rate $t(10) = 2.645$, $p = 0.025$). Male rats do not experience cyclic hormonal changes and as such, habitual male runners show stable running distances from day to day.

3.2.2. Gonadectomy

The impact of gonadectomy on distance run, time spent running, and rate of running was dramatic. Within one month after surgery and thereafter, ovariectomized females ran 12 times less distance than intact controls, a distance that was not over 1 km per day (intact and sham-operated were not different and so were pooled, $t(28.113) = 7.019$, $p < 0.001$). Orchiectomized males ran 30 times less distance than intact or sham-operated male controls (not over 0.5 km per day, $t(27.033) = 7.909$, $p < 0.001$). Overall, orchiectomized males spent significantly less (7%) of their time running (on average 0.81 h per 12-h dark period) compared to 36% (4.3 h) for control males, and ovariectomized females spent significantly less (10%) of their time running (1.2 h) compared to 31% (3.7 h) for control females (males, week 3, $t(28.734) = 12.210$, $p < 0.001$; females, week 3, $t(38.419) = 8.775$, $p < 0.001$). Gonadectomy also significantly reduced the daily rate of running, from the intact average of 13.5 m/min (males) and 19.2 m/min (females) to 3.0 m/min for orchiectomized males (week 3, $t(42) = 8.0141$, $p < 0.001$ and

Table 1
Impact of wheel access on microfeatures of running patterns.

	Time of Running and Dose of Wheel Access							
	1st Hour of Running				6th Hour of Running		12th Hour of Running	
	Full (*ad libitum*)	Half (alternate-day)	Limited (2-h)	Ultralimited (30-min)	Full (*ad libitum*)	Half (alternate-day)	Full (*ad libitum*)	Half (alternate-day)
Bout number	5.1 (±0.6)	4.0 (±0.4)	3.8 (±0.8)	1.0 (±0.0)	3.7 (±0.7)	2.3 (±1.0)	3.2 (±0.6)	5.3 (±1.7)
Bout time (minutes)	5.3 (±0.4)	5.5 (±1.3)	9.9 (±2.5)	30.0 (±0.0)	5.1 (±0.7)	6.3 (±1.2)	2.0 (±0.3)	5.0 (±0.6)
Bout distance (meters)	110.5 (±15.4)	90.3 (±31.2)	110.2 (±33.3)	586.5 (±46.4)	116.1 (±23.6)	142.5 (±32.8)	9.1 (±3.1)	99.1 (±16.3)
Bout rate (meters/minute)	15.2 (±1.1)	12.5 (±2.1)	8.0 (±1.2)	19.6 (±1.5)	15.9 (±2.0)	21.6 (±3.6)	2.8 (±0.5)	16.8 (±1.7)
Inter-bout interval (minutes)	5.5 (±0.7)	10.9 (±3.3)	6.5 (±2.0)	NA	10.6 (±2.0)	16.1 (±6.4)	14.4 (±2.5)	6.4 (±2.4)

Data are presented as average (±SEM). For the full-dose (*ad libitum*) group, data are averages of males and females whereas all other groups represent female data only. For the ultralimited runners, data presented here represent a more limited set of data than that presented in Table 2. This data was captured towards the end of the 3rd week of wheel access when ultralimited runners fully utilize the 30 min of wheel availability *versus* days 14–21 when they spend on average 82.4. ± 5.3 percentage of their time running (Table 2).

6.8 m/min for ovariectomized females (week 3, $t(54) = 11.3652$, $p < 0.001$). Gonadectomized animals continued to run in bouts, which were significantly different from their intact counterparts in terms of number (fewer), time (shorter), distance, and rate (less), each $p < 0.05$.

After gonadectomy, sex differences persisted and some even emerged. For example, similar to their intact counterparts, ovariectomized females ran significantly farther than orchiectomized males ($t(28.047) = 4.915$, $p < 0.001$). In addition, a sex difference emerged in that ovariectomized females spent significantly more time running than orchiectomized males ($t(35.133) = 2.853$, $p = 0.007$), while their respective intact comparisons did not differ in time spent running.

3.3. Characteristics of running with alternate-day wheel access; the half-dose running treatment

Regardless of whether rats had access to wheels every day for 3 weeks (full dose of running) or alternate days for 3 weeks, they ran the same distance and time such that at their peak, both groups spent 4 h running about 4.4 km/day (averaged across the days of the estrus cycle; Fig. 4A). Alternate-day access, however, affected the emergence of running, as well as the features of habitual running, including running rate and the light-dark cycle pattern of running (Fig. 4B; Table 1).

In subjects with consecutive-day access, peak distance was achieved in 2 weeks, but alternate-day females required 3 weeks to reach this same peak (week 1–2 $t(15) = -3.370$, $p = 0.004$; week 2–3: $t(15) = -2.171$, $p = 0.046$; Fig. 4A). Additionally, though consecutive-day runners required two weeks to reach their maximal running time, alternate-day runners reached their peak time running (4 h) during week 1 (week 1–2 $t(15) = -1.761$, $p = 0.99$). Consecutive- and alternate-day runners each reached their peak average running rate by week 2 (week 1–2 $F(1.15) = 33.931$, $p < 0.001$); however, thereafter, consecutive-day runners ran 22% faster than alternate-day runners (19.2 *versus* 15.7 m/min) ($t(50) = 2.487$, $p = 0.016$). Furthermore, the average and overall fastest rate of the consecutive-day runners were 30% (50 *versus* 38.5 m/min) and 28.2% (61 *versus* 47.6 m/min) faster than the alternate-day runners.

Our protocol choice to return the wheels to alternate-day runners during the light phase (1:00 PM, normally resting) revealed distinct running pattern differences between alternate- and consecutive-day runners (Fig. 4B)). Alternate-day runners ran significantly more in the light period than consecutive-day runners. It is typical for wheel running to occurs upon cage disturbance. For example, if cages are cleaned or food and water changed, rats will run a small amount of wheel turns during and shortly after this disturbance period. However, this is markedly different from wheel running that occurs in response to wheel return after a significant period of deprivation, such as 24 h. Immediately upon wheel return, alternate-day runners had a robust running response (170 m), which was significantly greater than normally resting consecutive-day runners left undisturbed in that hour (0 m). Additionally, at the end of the light period, alternate-day runners recommenced running sooner than consecutive day runners, presumably in anticipation of lights off and the usual peak running period.

Second, the pattern of running by alternate-day runners in the dark period was substantially different than consecutive-day runners. Unlike consecutive-day runners, running in alternate-day runners did not decrease during the dark period but rather remained stable, generally at a lower hourly rate (Fig. 4B). In the first 6 h, consecutive-day runners ran approximately 34% faster than alternate-day runners (22.7 *versus* 16.9 m/min, $t(50) = 3.654$, $p = 0.001$), covering 2.9 *versus* 1.8 km. For most of the second half of the dark cycle, consecutive-day runners ran at faster rates (18.4 *versus* 13.8 m/min, $t(50) = 3.226$, $p = 0.002$), but traveled a similar distance (~1.6 km). During the last hour of the dark period (Fig. 4B), consecutive-day runners performed very few wheel turns; however, alternate-day runners continued to be active, running 8 times more than consecutive day runners (323.5 *versus* 40.6 m) at a 3.5-times faster rate (12.9 *versus* 3.7 m/min, $t(50) = -7.661$, $p < 0.001$). For alternate-day runners, this level of running was maintained throughout the light period until the wheels were removed around 12:00 p.m.

The number of running bouts was the same for alternate- and consecutive-day runners (Table 1). For most of the dark period, the duration of the bouts and the distance run were also the same except in that remarkable last hour of the dark period during where alternate-day runners had bouts 2.5 times longer (5 min), achieving ~11 times the distance (99 m), compared to their consecutive-day counterparts, each $p < 0.05$.

A final point can be made about the difference in visibility of the distinct four-day cycling pattern (Fig. 3) from *the consecutive- versus alternate-day* runners, which had one of two patterns. If the alternate-day access occurred during proestrus and metestrus, significant changes could be seen in distance run from day to day (*i.e.*, due to comparison of proestrus *versus* metestrus), whereas if the alternate-day access occurred during estrus and diestrus, only moderate, seemingly stable levels were seen.

3.4. Characteristics of running with severely limited wheel access: the workout-dose running treatment

These experiments tested how severely limiting wheel access (2 h/limited *versus* 30 min/ultralimited per day) alters the onset of running as well as the characteristics of habitual running. Com-

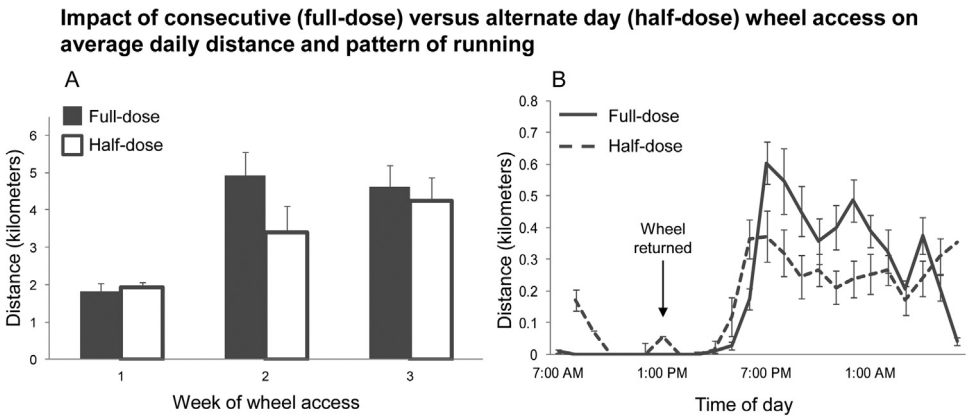

Fig. 4. Data are presented as average (±SEM). Impact of half-dose wheel access on running distances and patterns (A) Average weekly distance run during the first three weeks of wheel availability in full- and half-dose runners. Rodents ran similar distances during the first three weeks of wheel availability whether they were offered consecutive- or alternate-day wheel access. (B) Running patterns during the 24-h light/dark cycle in full- and half-dose runners. Despite running similar distances, patterns of wheel running activity were significantly different between groups. Compared to full-dose runners, half-dose runners ran at more consistent levels throughout the dark period, with a burst of running before and perhaps in anticipation of wheel removal.

pared to consecutive- and alternate-days runners, results suggest that many, but not all, features of the emergence and habitual phases of running were shaped by severely limiting the dose of daily running. Unlike full dose runners, females offered limited or ultralimited access to the running wheel did not show marked differences in daily running in a pattern that reflected the estrus cycle. That is, although they were cycling, their pattern of running was more shaped by their limited daily access to the wheel than by the stage of their estrus cycle.

3.4.1. Distance

Similar to full-dose wheel runners, rats with 30 min of daily access (ultralimited) reached their peak distance run by week 2, whereas rats with 2 h of daily access (limited) required an additional week to reach maximal running distances. Once running habits were established (by week 3; see Table 2), in a comparison that was proportional to the time the wheel was available (*i.e.*, the first 30 min of wheel availability), ultralimited runners ran significantly farther than either the limited- ($t(54) = 1.975$, $p = 0.053$) or full-dose runners ($t(54) = 2.822$, $p = 0.007$).

3.4.2. Time

The percentage of time that the rats spent running was inversely related to wheel availability. That is, the shorter the time the wheel was available, the larger percentage of time the rats spent running (Table 2) (ultralimited *versus* limited $t(54) = 4.283$, $p < 0.001$; limited *versus* full $t(44.854) = 2.663$, $p = 0.011$). By day 5, and consistently thereafter, ultralimited runners spent on average 82% of their wheel access time running, with some rats running 100% of their time. By week 3, limited runners reached an average of 49% of their time spent running, while full-dose runners spent only 31% of their time running.

3.4.3. Rate

Limiting wheel availability also markedly altered the overall rate of running (Table 2), with both ultralimited and limited subjects running at a slower average rate than those offered the full dose of wheel availability (ultralimited *versus* full $t(54) = -3.777$, $p < 0.001$; limited *versus* full $t(46.280) = -7.506$, $p < 0.001$). Additionally, the fastest rate achieved during wheel availability was significantly slower for both ultralimited and limited runners (ultralimited *versus* full $t(54) = -2.701$, $p = 0.009$; limited *versus* full $t(54) = -7.435$, $p < 0.001$).

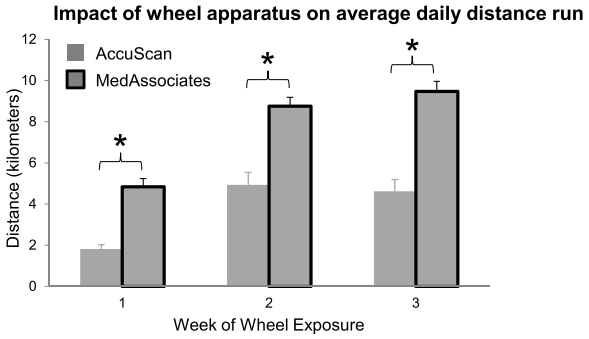

Fig. 5. Data are presented as average (±SEM). Impact of wheel apparatus on daily running distances. Distance but not pattern of the emergence and habitual phases of running are significantly altered by wheel running apparatus. * indicates a significant difference using an independent-samples *t*-test.

3.4.4. Bouts

Limiting wheel availability also dramatically altered bout running patterns. Specifically, ultralimited runners took no break in running, resulting in only 1 bout per 30-min period (ultralimited *versus* full $t(54) = 3.354$, $p = 0.028$). Thus, the bouts of ultralimited runners were 6.4 times longer (30.0 min), with animals running 5.8 times farther (587 m) than full-dose runners (Table 1).

3.5. Influence of different wheel apparatuses

Rats of comparable age, which were wheel naïve at the start of running, achieved stable running patterns, in terms of distance, time and rate, in a similar time frame (2 weeks) in either the AccuScan or Med Associates apparatus (Fig. 5) (AccuScan week 1–2: distance $F(1,27) = 21.472$, $p < 0.001$, time $F(1,27) = 15.084$, $p = 0.001$, rate $F(1,27) = 32.786$, $p < 0.001$; Med Associates week 1–2: distance $F(1,55) = 53.338$, $p < 0.001$, time $F(1,55) = 8.177$, $p = 0.006$, rate $F(1,55) = 53.680$, $p < 0.001$). Rats ran farther for significantly longer times at faster rates in the Med Associates apparatus than the AccuScan apparatus. At their peak (2 weeks and thereafter), compared to AccuScan runners, Med Associates runners traveled double the distance (9.5 km/day) ($t(64.268) = -6.436$, $p < 0.001$), spent 43% more time running (5.35 h/day) ($t(82) = -4.484$, $p < 0.001$), and ran at 1.5 times the rate (29 m/min) ($t(82) = -7.340$, $p < 0.001$). General time of day spent running and patterns of bouts were not different in the two wheel systems.

Table 2
Impact of wheel access on distance, time and rate of running.

Daily Dose of Running	Distance (meters)	Percentage of Time	Average Rate (m/min)	Average Fastest Rate (m/min)
Ultralimited (30-min)	398.8 (±41.2)[A,B]	82.4 (±5.3)[A,B]	14.5 (±1.3)[A,B]	30.5 (±5.5)[A,B]
Limited (2-h)	778.3 (±76.7)	48.9 (±5.8)[B]	10.8 (±0.7)[B]	27.6 (±1.8)[B]
	301.8 (±26.8)	81.0 (±4.4)	11.7 (±0.8)	23.1 (±1.4)
Full (*ad libitum*)	863.4 (±141.0)	30.8 (±3.6)	20.9 (±1.1)	46.0 (±1.7)
	226.8 (±45.0)	32.7 (±5.0)	16.1 (±2.1)	31.8 (±3.8)

Data are presented as average (±SEM). For the full-dose (*ad libitum*) group, data are averages of males and females whereas all other groups represent female data only. For the limited-dose group, the first row represents the average for all 120 min of data, whereas the second row represents the average for the first 30 min of data (*i.e.*, a proportional comparison to ultralimited runners). For the full-dose (*ad libitum*) group, the first row represents a proportional comparison to limited runners taken at a similar period of the dark cycle, whereas the second row represents a proportional comparison to ultralimited runners. A = Significant difference to limited dose using an independent-samples *t*-test; B = Significant difference to full dose using an independent-samples *t*-test. For distance, statistical comparisons were performed between ultralimited and 30-min proportional comparisons. For all other variables, statistical comparisons were performed between ultralimited and two-hour comparisons.

Further, the distances run were dependent upon continued access to the same type of apparatus. That is, subjects with three weeks of initial exposure to the Med Associates apparatus, significantly slowed running in their fourth week of running if placed in an AccuScan apparatus ($F(1,27) = 32.456$, $p < 0.001$). Similarly subjects with three weeks of initial exposure to AccuScan, significantly increased running by 1.6 times and restabilized to this higher rate in subsequent weeks of running if placed in a Med Associates apparatus (($F(1,6) = 20.601$, $p = 0.004$).

4. Discussion

Our results provide a roadmap of considerations for investigators who are planning to use wheel running as an independent variable or intervention in service of driving changes in their dependent variables of interest. Features of this work include both confirmation of previous hypothesis using modern quantitative methods, as well as novel data and directions. In addition, these data demonstrate the importance of examining the microfeatures of running, rather than simply looking at total distance run; certain new wheel running systems are being developed to assess these intricate details of the behavior (Chomiak et al., 2016).

4.1. Wheel naïve rats take several weeks to develop stable running behavior

In agreement with previous reports, the present data show that to produce stable maximal running distances, naïve rats require up to 3 weeks of *ad libitum* wheel access (Afonso and Eikelboom, 2003; Eayrs, 1954; Eikelboom and Lattanzio, 2003; Eikelboom and Mills, 1988; Greenwood et al., 2011; Lattanzio and Eikelboom, 2003; Looy and Eikelboom, 1989; Mueller et al., 1997; Richter, 1927). We additionally uncovered an effect of sex, finding that females reached their peak a week earlier than males (2 *versus* 3 weeks). In addition, shorter periods of wheel access lengthened the time to emergence of stable, maximal running by a week, as demonstrated by the half-dose and limited access protocols. In contrast, ultralimited runners only took two weeks to reach their maximal levels, due to the theoretically smaller gains in physical fitness required to run for this length of time.

These data suggest that paradigms using the first two to three weeks of running to induce changes in a dependent variable are doing so prior to the achievement of stabilized, maximal running. Researchers should thus be aware that outcomes produced during this period may be affected by variable rates of acquisition and do not represent the effects of chronic exercise *per se*. Protocols that use three weeks of running or more produce subjects in which daily running has stabilized, avoiding this potentially "hidden" source of variation.

4.2. Positive incentive salience of the wheel can be used as an important force to regulate running

As compared to *ad libitum* wheel access, alternate day, limited, and ultralimited wheel availability result in changes in the daily running pattern, with such changes occurring by the third day of wheel availability. Rats quickly learn that wheel availability is temporally restricted, and they adjust their running rates to obtain a particular amount of running within the allotted time frame. We conclude that these altered behaviors are due to the positive incentive salience of wheel running (Basso and Morrell, 2015; Belke and Wagner, 2005; Greenwood et al., 2011; Lett et al., 2000; Lett et al., 2002), and suggest that it can be used to even further modify the subject's running pattern.

Rats given ultralimited or limited wheel access altered the time, rate, and bouting pattern of running. These groups spent a greater percentage of their time running (82% in ultralimited and 49% in limited *versus* 30% *ad libitum* dark period), but had ~25% lower running rates than the *ad libitum* access group. Additionally, ultralimited runners had stable running rates during the 30 min, whereas limited runners decreased their running rate during the wheel availability time, even during the first 30 min. These results indicate that limiting wheel access enhances the motivation for running, but results in slower and possibly sub-maximally fit rodents.

Previous studies have shown that rebound running or significant increases in running behavior occur after periods of forced wheel deprivation (Basso and Morrell, 2015; Mueller et al., 1999; Mueller et al., 1997). Our data additionally show that alternate-day wheel access produces a distinct pattern of running in which rats maintain the same total distance per 24 h but adjust the pattern within the cycle. Specifically, rats run stably throughout the majority of the dark cycle and continue to run until the wheel is removed. This indicates that rats establish a set point for the amount of daily running and fine-tune their behavior to achieve this set point when wheel availability is restricted. This intriguing phenomena is without any currently known mechanism. We postulate that such motivational processes require the capacity of learning and memory circuits along with prefrontal executive planning and execution systems (Basso and Morrell, 2015).

4.3. Age at first wheel exposure does not influence acquisition or habitual levels of running

In our experiments, rats offered wheel access early in life (prepubertal juveniles) showed similar acquisition patterns of running as rats offered wheel access as adults. Additionally, stable, habitual levels of running were similar between these two groups. This is in contrast to experiments conducted by Looy and Eikelboom showing that male rats introduced to the wheel at an older age (over 100 days of age) did not run significant daily distances or increase

their daily distance run over time (Looy and Eikelboom, 1989). Our findings were surprising as we speculated that younger runners would take a longer time to develop maximal levels of running. This indicates that both the physical capacity as well as the motivation for voluntary wheel running develops at an early age. Investigators interested in the interaction between exercise and brain development should be aware that maximum running can be achieved even at these early time points.

4.4. Weight gain reduces the amount of running even after maximal, stable running is achieved

When animals are typically 4 months of age or younger and weigh less than 500 g, no correlation is seen between body weight and distance run, regardless of sex. Cycling females display modest weight gains and continue with robust stable, maximal running for up to six months. Male subjects, however, gain more weight during this time period and subsequently run markedly less. Thus, in males, the gradual decrease in running may be a "hidden" or unintended variation in the independent variable of running behavior. Another interpretation of this decrease in daily running distances in growing males is discussed in Section 4.8 below.

4.5. Sex differences in distance run and rate of running suggest that females may be a better model to maximize changes in the dependent variable of interest

Once stable, maximal running is established, females run on average 1.5 greater distances than males, which they do by running significantly more during the second half of the dark cycle. This finding is in accord with the limited available literature, which contains few systematic comparisons across the sexes (Afonso and Eikelboom, 2003; Eayrs, 1954; Eikelboom and Lattanzio, 2003; Eikelboom and Mills, 1988; Lattanzio and Eikelboom, 2003; Richter, 1927).

Our data demonstrate that the greater distance run by females is due to their faster running rate, which is in accord with (Eikelboom and Mills, 1988) (the only prior measure of the effect of sex on rate). Additionally, when examining running at the level of bouting, we see that though the number of bouts is similar across sexes, the duration of the bouts, the distance run per bout, and the rate of running during the bout is higher in females (Eikelboom and Mills, 1988).

The fact that females routinely run 10–15 kilometers (6–9 miles) and males 3–8 kilometers (2–5 miles) per day is of critical importance. Although the precise mileage may vary in other settings, possibly due to running apparatus or strain of rat, we posit that cycling females of any given strain will run close to twice the distance as males by running at substantially faster rates.

We speculate that the bulk of the running wheel literature in rodents uses males so to avoid the effects of the estrus cycle. Our data suggest, however, that if an experimental goal is best met by maximal running capacity or many months of stabilized running, female subjects may well be a better choice. If males are chosen, maximal running is likely to decrease more quickly as body weight increases at a faster rate than females. Food restriction, while offering the appeal of keeping the males lighter, is likely going to tap into brain mechanisms that add complications to the variables at work (Chowdhury et al., 2015; Ho et al., 2016; Klenotich and Dulawa, 2012; Routtenberg and Kuznesof, 1967).

4.6. Effects of estrus cycle on wheel running

By carefully tracking cyclic changes in vaginal cell cycle stage along with the daily running totals, we were able to delineate the features of running during the entire hormonal cycle. In general, the features of this pattern can be seen after one week of wheel exposure, although it is more fully developed after 2 weeks, when intact females have fully stabilized running. This tracking allowed us to readily predict the day of the estrus cycle, without the need for constant contemporaneous histological verification of cycle stage. Thus, in any given data set, after minimal cycle verification by histology, the reliable changes in running allows for predictive or retrospective choices in choosing data points at desired stages of the cycle. Previous studies have shown that ovarian hormones significantly affect voluntary wheel running, with drastically different daily distances seen across the estrus cycle; the greatest distances occur during proestrus (Richter, 1927; Carmichael et al., 1981; de Kock and Rohn, 1971; Long and Evans, 1922; Slonaker, 1924; Wang, 1923). Our data confirm those findings, showing that from metestrus to proestrus, the daily distance doubles, which is accomplished through substantially longer running times than males (or females in metestrus) and faster average running rates. The same trend can be seen at the level of the running bouts; proestrus females run in longer bouts that occur at faster rates. We further show that the shortest distance run by females (metestrus) is similar to the maximal amount run by males.

Along with the proestrus data, the fact that metestrus running patterns mimic male running patterns (up to a body weight of 500 g) suggests that sex differences in voluntary wheel running behavior (at least before 15 weeks of age) are more heavily influenced by daily changes in the hormonal cycle rather than by body weight or muscle mass/tone. We speculate that contemporaneous circulating gonadal steroid hormones alter particular CNS processes that are the core source of such differences in running. Such effects might be due to the action of steroid hormones on neuronal activity *via* their known receptors in the striatum, possibly including both the nuclear receptors and membrane receptors, which are speculated to participate in rapid effects of gonadal steroid hormones across the cycle (Becker et al., 1987; Mermelstein et al., 1996; Morrell et al., 1995).

4.7. Circulating adult gonadal hormones are the principle but not sole basis of sex differences in running

Previous reports revealed that gonadectomy of adult females, and the few studies that included males, reduced daily running distance by 60–95%. Notably, ovariectomized females show a flat pattern of daily wheel running, with complete disappearance of the 4-day rhythmic running cycle (Gerall et al., 1973; Richter, 1927). We confirmed prior findings in both sexes, without the housing and apparatus confounds likely in the Richter (Richter, 1927) study, and further demonstrated that removal of the gonadal hormones dramatically decreased distance run by decreasing time spent running and running rate at all time points by approximately 90%, with dramatic alterations in all features of bouting. Gonadectomy did not affect the pattern of running over the light-dark cycle, but blunted the bout pattern compared to intact males and females, a finding not previously reported. Additionally, sex differences in voluntary wheel running survive gonadectomy, such that ovariectomized females still run farther by running faster and for longer periods of time than orchiectomized males. This indicates, as others have theorized before (Sherwin, 1998), that voluntary wheel running is not solely regulated by contemporaneously circulating gonadal hormones.

Earlier studies allowed rats to begin running almost immediately after gonadectomy; however, we now know that behavioral changes from gonadectomy or steroid hormone replacement therapy in gonadectomized subjects requires several weeks to manifest (Sachs and Meisel, 1988). Thus, prior studies likely included data points before complete washout of the effects of the hormones. Assuming that in these prior studies gonadectomies were complete,

this likely explains why a previous report (Richter, 1927) found that substantial running occurred after gonadectomy. In contrast, we allowed hormone levels and hormone-dependent peripheral and brain effects sufficient time to decrease before running measures recommenced.

4.8. Effects of wheel apparatus on running behavior

We utilized two different wheel systems to measure a variety of wheel running parameters. These systems differed in distinct ways including diameter of the wheel, width of the running track, and the materials of the wheel itself (mesh wire *versus* steel bars). Compared to the AccuScan system, the MedAssociates system is larger in both diameter and width, and its mechanical design allows for a smoother turning of the wheel. When we look at daily distance run, rats ran significantly more in the MedAssociates system. This could indicate that the animals ran less in the AccuScan wheels due to some less preferred aspect of the size and structure of the wheel; simply put, the rodents were less comfortable in the AccuScan wheels. This interpretation would also account for the fact that male rats in the AccuScan system significantly decrease their running as they gain both length and weight. Indeed, Looy and Eikeboom found that male rats who were introduced to the wheels when they were approximately 300-315 g, maintained running over the next 70 days (500 g and above) and ran significant distances of 3–5 km per day (Looy and Eikelboom, 1989), suggesting that their experimental subjects found the size and structure of their wheels more suitable/comfortable. Taken together, our data with that of Looy and Eikelboom suggest that it is important to track 1) gender, 2) strain of subject, 3) running wheels utilized, and 4) body weights throughout the experiment, as these variables can result in considerable differences in daily distance run, which may impact the dependent variables under investigation.

4.9. Additional variables that affect wheel running

Though we have explored many different variables that influence the behavior of wheel running, this is by no means an exhaustive list. Many other factors influence wheel running such as food and/or water deprivation, presence of pups or a sexual partner, availability of other enrichment objects, or time of day the wheel is provided (*i.e.*, day *versus* night access). In addition, endless running wheel protocols are possible, which may uniquely influence the behavior of wheel running. As an example, another way to administer what we have referred to as the "half-dose" running, would be to consider it from a daily perspective and provide the wheels only during the 12-h dark cycle. We posit that the running pattern would look similar to that of *ad libitum* access, and hence not really be our intended "half-dose"; we considered great complications would arise from providing the wheel at some time period that was antithetical or partially antithetical to the rats' strong propensity to run mostly in the dark. What would happen if the running wheel was provided in a limited but random and unexpected manner? Could this increase the motivation to wheel run even more? Future experiments using new running wheel protocols will need to investigate how their unique dose of wheel running influences the behavior of wheel running.

4.10. A rat's eye view of the human condition

Currently, 80% of adults in the United States do not achieve the level of physical activity recommended by the American Heart Association, while 25% are not at all physically active, resulting in a pressing public health problem. Indeed, a sedentary lifestyle is one of the leading causes of death (Johnson et al., 2014; Mokdad et al., 2004). We agree with Eikelboom (Eikelboom, 1999) that voluntary wheel running studies in rodents have potential as a preclinical model for exercise in humans. To this, we add that our data on the "workout doses" of wheel running suggest that shortened workout regimes do not provide the same conditioning as can be found even with somewhat longer regimes, and that the reduced conditioning found in subjects with greater body weight and lower circulating levels of gonadal hormones suggest additional challenges in the human situation. Hope is offered by the idea that exercise has positive incentive salience, which may serve to increase the motivation to engage in this healthy behavior.

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