6-4-2018

The Effects of Volume Versus Intensity of Long-Term Voluntary Exercise on Physiology and Behavior in C57/Bl6 Mice

Lisa S. Robison

Dominique L. Popescu

Maria E. Anderson

Steven I. Beigelman

Shannon M. Fitzgerald

See next page for additional authors

Citation/Publisher Attribution

Robison, L.S., Popescu, D.L., Anderson, M.E.,Beigelman, S.I., Fitzgerald, S.M., Kuzmina, A.E., Lituma, D.A.,...Robinson, J.K. (2018). The effects of volume versus intensity of long-term voluntary exercise on physiology and behavior in C57/Bl6 mice. *Physiology and Behavior*, *194*, 218-232. doi: 10.1016/j.physbeh.2018.06.002
Available at: https://doi.org/10.1016/j.physbeh.2018.06.002

The Effects of Volume Versus Intensity of Long-Term Voluntary Exercise on Physiology and Behavior in C57/Bl6 Mice

Authors

Lisa S. Robison, Dominique L. Popescu, Maria E. Anderson, Steven I. Beigelman, Shannon M. Fitzgerald, Antonina E. Kuzmina, David A. Lituma, Sarima Subzwari, Michalis Michaelos, Brenda J. Anderson, William E. Van Nostrand, and John K. Robinson

Physiology & Behavior 194 (2018) 218–232

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/physbeh

The effects of volume *versus* intensity of long-term voluntary exercise on physiology and behavior in C57/Bl6 mice

Lisa S. Robison[a,*,1], Dominique L. Popescu[a], Maria E. Anderson[a,2], Steven I. Beigelman[a], Shannon M. Fitzgerald[a], Antonina E. Kuzmina[a], David A. Lituma[a], Sarima Subzwari[a], Michalis Michaelos[a,3], Brenda J. Anderson[a], William E. Van Nostrand[b,4], John K. Robinson[a,5]

[a] Department of Psychology, Stony Brook University, 100 Nicolls Road, Stony Brook, NY 11794, United States
[b] Department of Neurosurgery, Stony Brook University, 100 Nicolls Road, Stony Brook, NY 11794, United States

ARTICLE INFO

Keywords:
Aerobic exercise
Cardiovascular exercise
Anxiety
Cognition
Memory

ABSTRACT

Cardiovascular exercise (CVE) is associated with healthy aging and reduced risk of disease in humans, with similar benefits seen in animals. Most rodent studies, however, have used shorter intervention periods of a few weeks to a few months, begging questions as to the effects of longer-term, or even life-long, exercise. Additionally, most animal studies have utilized a single exercise treatment group – usually unlimited running wheel access – resulting in large volumes of exercise that are not clinically relevant. It is therefore incumbent to determine the physiological and cognitive/behavioral effects of a range of exercise intensities and volumes over a long-term period that model a lifelong commitment to CVE. In the current study, C57/Bl6 mice remained sedentary or were allowed either 1, 3, or 12 h of access to a running wheel per day, 5 days/weeks, beginning at 3.5–4 months of age. Following an eight-month intervention period, animals underwent a battery of behavioral testing, then euthanized and blood and tissue were collected. Longer access to a running wheel resulted in greater volume and higher running speed, but more breaks in running. All exercise groups showed similarly reduced body weight, increased muscle mass, improved motor function on the rotarod, and reduced anxiety in the open field. While all exercise groups showed increased food intake, this was greatest in the 12 h group but did not differ between 1 h and 3 h mice. While exercise dose-dependently increased working memory performance in the y-maze, the 1 h and 12 h groups showed the largest changes in the mass of many organs, as well as alterations in several behaviors including social interaction, novel object recognition, and Barnes maze performance. These findings suggest that long-term exercise has widespread effects on physiology, behavior, and cognition, which vary by "dose" and measure, and that even relatively small amounts of daily exercise can provide benefits.

1. Introduction

Cardiovascular exercise (CVE) can mitigate the risk of several diseases and negative health outcomes. CVE is protective against cancer and obesity-related disorders (*e.g.*, type II diabetes mellitus, stroke, osteoarthritis), and improves cardiopulmonary function and sleep [1]. Psychologically, exercise is linked to several mental health benefits, including enhanced mood, and reduced stress, anxiety, and depression [2–4]. Cognitive benefits have also been noted, including enhanced learning and memory, and protection against cognitive decline during aging and Alzheimer's disease [1, 5, 6]. Despite overwhelming evidence of the positive benefits of CVE, an optimal "dose" of exercise to achieve maximum health benefits, let alone cognitive benefits, remains far from determined.

It has been well documented that higher levels of CVE are associated with improved functioning in older adults [5, 7–9]. Early cross-

* Corresponding author.
E-mail address: robisol@amc.edu (L.S. Robison).
[1] Current Address: Department of Neuroscience and Experimental Therapeutics, Albany Medical College, 47 New Scotland Avenue, Albany, NY 12208.
[2] Current Address: Department of Psychology, Farmingdale State College. 2350 Broadhollow Road, Farmingdale, NY 11735.
[3] Current Address: Janelia Research Campus. 19700 Helix Drive, Ashburn, VA 20147.
[4] Current Address: George & Anne Ryan Institute for Neuroscience and Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, 130 Flagg Road, Kingston, RI 02881.
[5] Current Address: George & Anne Ryan Institute for Neuroscience and Department of Psychology, University of Rhode Island, 130 Flagg Road, Kingston, RI 02881.

https://doi.org/10.1016/j.physbeh.2018.06.002
Received 29 January 2018; Received in revised form 30 May 2018; Accepted 1 June 2018
Available online 04 June 2018

sectional studies found that individuals who exercised performed better than non-exercisers across a number of cognitive domains, including response time, reasoning, memory, vigilance, fluid intelligence and exhibited better performance on the Stroop and Trail-making tasks [10–14]. Results from longitudinal studies are also positive, linking mid-life physical activity to reduced risk of cognitive decline and dementia in later life [15, 16]. In a study of healthy older adults, three days/week aerobic exercise increased memory performance compared to a stretching control group [8]. In a randomized controlled trial, 24 months of moderate intensity exercise had no effect on cognitive performance in previously sedentary older adults [17], suggesting that earlier, longer, or even life-long exercise interventions may be necessary to be effective. Importantly, these studies suggest that CVE may lead to physiological changes that alter the course of cognitive decline.

Studies in rodents have yielded similarly positive results, such that CVE is capable of improving learning and memory across various ages [18–22]. These studies typically employed either voluntary exercise interventions on running wheels or forced exercise on treadmills; however, a majority of these studies provided unlimited wheel access, resulting in large volumes of exercise not comparable to the human clinical scenario. While the methods outlined in these studies vary in the exercise-duration parameters, few systematically compared the effects of different amounts of exercise. In fact, exercise was typically presented as a treatment that was compared only with a sedentary control condition and did not assume that the intervention may have potential dose-response properties.

There are two important considerations then for both human health and the modeling of exercise in rodents. The first is to determine how different amounts of CVE affect physiological and behavioral measures. It may be that more is better, or alternatively, that an optimal level exists. Some evidence can be found in epidemiological studies showing that greater physical activity levels are linked to enhanced cognitive performance in healthy older women [23]. A meta-analysis found that exercise session durations of longer length (30–60 min) were more effective than shorter durations (15–30 min) in improving cognitive function [5]. In contrast, some studies suggest that only small doses of exercise are necessary to see significant health benefits [24, 25], and there is evidence that shorter duration high-intensity regimens are equally beneficial to longer-duration/lower-intensity regimens in regard to cardiovascular and metabolic function and musculoskeletal benefits [26–29]. Assessment of exercise regimens of varying intensity in rodents has generally been performed using forced exercise (treadmill running) [30–32], which is a known stressor and argued to be more stressful than voluntary wheel running [33–36]. Therefore, it is of interest to create voluntary exercise regimens that naturally vary in volume and intensity in animal models.

The present study assessed the “dose-dependent” effects of exercise (one hour, three hours, or 12 h of daily running wheel access) compared to sedentary controls, providing the opportunity to assess how different exercise patterning affected behavior and physiological outcomes. We hypothesized that providing mice with differing lengths of access to a running wheel might result in differences in exercise volume (wheel rotations), as well as measures that could be indicative of higher running “quality”/“intensity”. Animals with shorter access to a running wheel may run faster and/or take fewer breaks in order to maximize running output per available unit time; conversely, mice with longer access periods may achieve more cumulative running but take more frequent or longer rest breaks. These different patterns of exercise structure could presumably result in very different physiological adaptations and behavioral consequences. The second important consideration not represented in prior rodent studies is the effects of a lifetime of CVE. Virtually all previously mentioned exercise studies in rodents have had interventions lasting four months or less, while some clinical findings suggest that longer, or even life-long, exercise interventions may be necessary to be effective [17]. A meta-analysis also found that intervention length was a factor in the efficacy of exercise to exert beneficial effects [5]. Therefore, the current study utilized an eight-month intervention, assessing the impact of longer-term treatment. In sum, this study represents the first long-term intervention study examining different daily levels of exercise to model a life span of regular aerobic exercise in a human on physiological response measures and a comprehensive behavioral battery assessing motor function, temperament and cognition.

2. Materials and methods

2.1. Animals

Forty C57/Bl6 mice were used in this experiment, split equally between males and females (Taconic Biosciences, Rensselear, NY) and housed individually for the duration of the experiment in a controlled room (22 ± 2 °C and 40–60% humidity) with a 12 h reverse light-dark cycle (lights off 0800 h). C57/Bl6 mice were chosen as they are a common background strain of many transgenic mouse models of human disorders. Additionally, studies comparing running and its effects in several mouse strains found that C57/Bl6 mice run moderate amounts compared to other strains (~4 km/day) when given unlimited wheel access, which results in increased hippocampal neurogenesis and dentate gyrus volume, as well as improved cognitive performance [37].

Mice were allowed to habituate to a new housing facility and single housing for at least one week prior to the start of the experiment, referred to as the “pre-intervention” period. Baseline food intake and body weight measures were taken at the end of this period. Mice were then split into four experimental groups ($n = 10$/group): sedentary (Sed), one hour of wheel access/day (1 h), three hours of wheel access/day (3 h), and twelve hours of wheel access/day (12 h). Purina Lab Diet chow was available *ad libitum* for the entire experiment, and body weight and food intake were recorded weekly throughout the entire experiment. All experiments were conducted in conformity with the National Academy of Sciences Guide for Care and Use of Laboratory Animals and approved by the Stony Brook University Institutional Animal Care and Use Committee.

2.2. Voluntary exercise intervention

A timeline of the experiment is shown in Fig. 1. Exercise intervention began at 3.5–4 months of age. The intervention lasted for approximately 8 months, followed by behavioral testing, and mice being euthanized at ~12–13 months of age. Throughout the intervention period, mice were given access to a running wheel for five days per week during the dark cycle. Exercise mice were placed in 43 × 21 × 21 cm cages equipped with metal running wheels for the appropriate length of daily access (1 h, 3 h, or 12 h). The running wheel measured 6.5 in. in diameter with a 3 in. wide running platform. Number of rotations were recorded through a software system that responded to the closing of a switch produced by two magnets lining up – one magnet located directly on the wheel, and another on the outside of the cage. Sedentary animals remained in their home cages during this time, as has been done in previous studies examining the effects of exercise on brain and behavior [38]. Voluntary wheel running was chosen over forced exercise, such as a treadmill running regimen, so that mice could choose when and how much to run. This minimized potential confounding variables introduced by forced exercise, such as stress [33–36] and allowed for the correlation between running and other measures.

Running behavior was recorded during each session to determine total daily exercise, as well as investigate patterns of activity throughout sessions. The number of revolutions performed in each minute of wheel access was recorded. Running behavior was investigated to assess differences in volume and intensity between exercise groups and determine their possible role in affecting physiology and behavior. Average number of total revolutions per session was

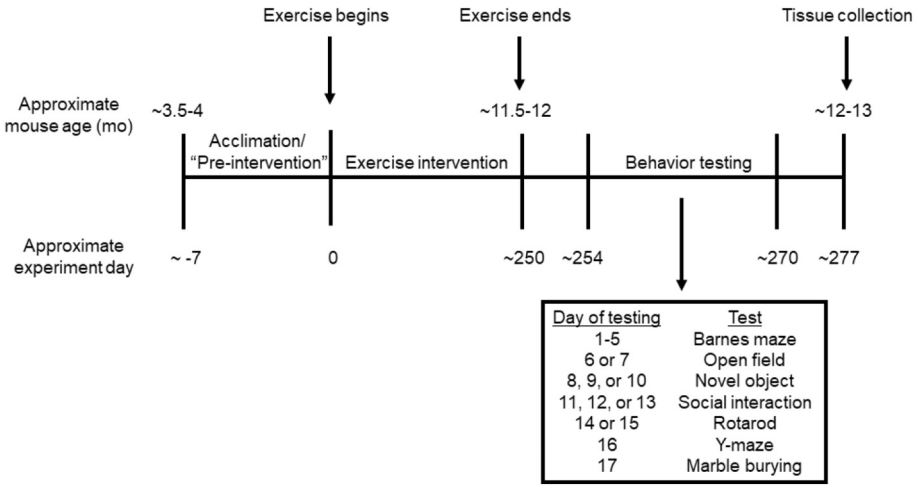

Fig. 1. Timeline of the experiment.

calculated to determine running volume. Average rotations/min (for binned minute with > 5 rotations) and breaks/h (number of minute bins with < 5 rotations) were calculated as measures of running intensity.

2.3. Behavioral assessment battery

All mice underwent a battery of behavioral testing following the eight-month intervention period, including rotarod, open field, social interaction, marble burying, novel object recognition, Y-maze for spontaneous alternation, and Barnes maze. Exercise interventions were not continued throughout the behavioral testing period, with testing beginning four days after the last exercise session. This allowed for the investigation of the effects of chronic (rather than acute) exercise. All behavioral testing occurred during the animals' dark cycle. Tests were performed in the following order, with no mouse receiving more than one behavioral assay in the same day: Barnes Maze (Days 1–5), open field (Day 6 or 7), novel object recognition (Day 8, 9, or 10), social interaction (Day 11, 12, or 13), rotarod (Day 14 or 15), Y-maze (Day 16), marble burying (Day 17).

2.3.1. Rotarod

Rotarod was performed to assess balance, strength, and motor coordination. The apparatus (model ENV-575 M; MED Associates Inc.) is composed of a 30 cm long rod that is divided into five, equally sized 6 cm sections. Mice were placed on the rotarod, which spun at an increasing speed of up to 40 rpm over a five-minute period. The time spent on the rod until the mouse fell (maximum time of five minutes) was recorded. Mice were tested three times, with a minimum five-minute inter-trial interval, and the average of the best two trials was used for analysis.

2.3.2. Open field

Mice were placed in a square 60 cm × 60 cm open field arena for 10 min. Behavior was recorded using ANY-maze software. Locomotor behavior was investigated with the measure of distance traveled, used to assess general activity levels and determine differences in motor function. Since mice tend to avoid open spaces and prefer exploring close to the walls of the open field apparatus (*i.e.* thigmotaxis), anxiety-like behavior was assessed by time spent in the center of the open field as in previous studies [39]. Measures of general activity and anxiety-like behavior are also useful in interpretation of any differences in performance observed in the Barnes maze.

2.3.3. Social interaction

Crawley's three chamber paradigm was adapted to assess sociability [40]. The apparatus used is a rectangular box measuring 46 cm × 21 cm, which consisted of three connecting compartments made of clear plexiglass walls. The side chambers measured 16 cm × 21 cm, and the middle chamber measured 14 × 21 cm. This test consisted of two five-minute trials that occurred consecutively, with the first trial serving as habituation to the chamber. In the second trial, the middle chamber was empty, one side chamber contained an empty cup, and the other side chamber housed another mouse in an identical cup (Stranger 1). Location of Stranger 1 (left or right side) was randomly assigned. Sociability was assessed by comparing time spent with Stranger 1 compared to the empty cup. Stranger mice were previously habituated to being confined in the cup within the arena to reduce distress during experimental trials.

2.3.4. Marble burying

Experimental procedures were adapted from a well-defined protocol [41]. Mice were placed in a rat-sized tub cage filled with 5 cm of corn cob bedding and 20 marbles in a 5 × 4 array for five minutes, during which time the latency to dig, number of digging episodes, and time spent digging were recorded. Digging was defined as coordinated movements of fore or hind limbs that displaced the bedding.

2.3.5. Novel object recognition task

A novel object recognition task was performed to assess object recognition memory. This task consisted of two trials, each lasting five minutes, with an inter-trial interval of 15 min. In the first trial, two of the same objects were placed in the open field arena, one in the front left quadrant, and one in the back right quadrant. In the second trial, one of the objects was replaced by a novel object, while the other object remained the same. Novel object recognition was assessed by the time spent with the novel object compared to the object used for the previous trial (familiar object).

2.3.6. Y-maze for spontaneous alternation

Mice were placed in a Y-shaped maze consisting of three arms. This maze was adapted from the unreinforced radial arm maze by blocking off five of the eight arms. Animals were allowed to freely explore the arms for three minutes, and number of arm entries was recorded. Order of arm entries were also manually assessed, and percent alternation was calculated [# alternations/(# arm entries − 2) ∗ 100]. Chance performance for continued alternation is 22.2%. In healthy exploration, mice should alternate traversing down arms in a circular pattern rather than repeatedly going down the same arms, based on the natural tendency of mice to explore novel environments. One alternation consisted of a mouse going down each of the three arms before returning to a previously-visited arm. Performance on this task is interpreted as a

measure of spatial working memory [42].

2.3.7. Barnes circular maze

The Barnes maze was originally developed to test learning and memory in rats (Barnes, 1979). We used an adaptation of this maze, a circular wooden platform, 91 cm in diameter, elevated 75 cm off the ground. The platform has eight equally spaced escape holes along the periphery that are 24.5 cm apart. Under each hole, a shelf securely held an escape box, measuring 10 cm × 8.5 cm × 4 cm. There were visible distal cues placed around the room, which remained constant throughout the duration of testing. Testing was performed on five consecutive days, with two trials per day separated by a 15 min inter-trial interval. Mice were placed onto the center of the maze at the beginning of each trial, then allowed to explore until the escape box was found and entered, or a maximum of five minutes. If the escape box was entered, the mouse remained there for one minute before being transferred back to its home cage. If escape box was not entered within five minutes, the mouse was placed in the escape box and left there for one minute. During each trial, the following measures were recorded: latency to find (amount of time taken to find escape box hole), hole entry rate (average time between subsequent hole entries as a measure of exploration speed), errors (number of nose-pokes into a hole that did not contain the escape box), and re-entry errors (number of nose-pokes into a previously visited hole that did not contain the escape box).

2.4. Physiological measures

2.4.1. Blood, organ, and muscle collection

Approximately one week following the completion of behavioral testing (~3–4 weeks after the completion of exercise), mice were euthanized between 1100 h and 1700 h under deep anesthesia with 2.5% avertin. Cardiac puncture was performed to collect blood, which was allowed to clot at room temperature for 30 min, spun at 2000 ×*g* for 10 min, and serum was collected and stored at -80 °C until used in assays. Wet organ weights were taken of the brain, heart, lungs, kidneys, liver, spleen, pancreas, and adrenal glands, which were also normalized by body weight. Additionally, quadriceps, gastrocnemius, and soleus muscles were collected to assess exercise-induced differences in muscle mass.

2.4.2. Citrate synthase activity in muscle

Muscles collected during euthanasia were assayed for citrate synthase activity, which has been used as a marker of aerobic capacity and mitochondrial density in skeletal muscle [43]. Muscle samples were added to 20 mL of CelLytic MT mammalian tissue lysis/extraction reagent (Catalog Number C3228, Sigma Aldrich) per gram of muscle tissue and homogenized using Zirconium Oxide beads and a bullet blender at 4 °C. Samples were spun at 16,000 ×*g* for 15 min at 4 °C, and supernatant was collected and stored at -80 °C until assayed for citrate synthase activity using a commercial kit (Catalog Number CS0720, Sigma Aldrich) according to the manufacturer's instructions. Citrate synthase activity was normalized by protein content, as done previously [44].

2.4.3. Enzyme linked immunosorbent assay (ELISA) for serum corticosterone

Serum samples were analyzed using a commercially available ELISA for corticosterone according to the manufacturer's instructions (Cayman Chemical). Absorbance was recorded using a plate reader (Spectramax).

2.5. Statistical analyses

One way ANOVAs were performed to determine differences between treatment groups on running parameters, body weight gain, overall food intake, organ and muscle mass, serum corticosterone concentration, and behavioral measures. Two-way repeated measures ANOVAs were performed to assess differences in treatment groups over time for running parameters, and food intake and body weight. Two way repeated measures ANOVAs were also performed to assess differences in exploration of objects (during the novel object recognition task) or cups (during social interaction) between treatment groups. When appropriate, Pearson correlations were performed to assess relationships between running parameters and behavioral and physiological measures. Analyses were performed using Statistica and SigmaPlot/Stat, and significance was set at alpha $p < .05$.

3. Results

Mice were subjected to 8 months of either sedentary or exercise conditions, with exercise groups being given either 1, 3, or 12 h of access to a running wheel (based on treatment group) 5 days per week. Mice then underwent an extensive battery of behavioral testing to characterize the effects of exercise on motor function, temperament, and cognition. Following behavior testing, mice were euthanized to collect blood, organs, and muscles for assessment of physiological outcomes. The current study included both males and females; however, separating analyses by sex results in an abundant loss of power. Because sex differences have been reported previously in voluntary running patterns and behavioral responses to running [*e.g.* [45, 46]], symbols were added to figures to allow preliminary evaluation of these presently.

3.1. Running parameters

3.1.1. Rotations

The number of rotations performed by each mouse was recorded with computer software in one-minute bins during each exercise session. The total number of rotations performed was summed during each session as a measure of running volume. A two way repeated measures ANOVA was performed to determine the effect of treatment on average rotations performed per exercise session during each month of the intervention (Fig. 2A). There was a significant main effect of treatment [$F(2, 27) = 79.580$, $p < .001$], with post-hoc analyses showing that mice with longer access to a running wheel performed a greater number of rotations (12 h > 3 h > 1 h; $p < .001$ for all). The main effect of time was significant [$F(7,189) = 32.264$, $p < .001$], with a trend of mice running less as time went on during the experiment. The treatment × time interaction was also significant [$F(14,189) = 10.129$, $p < .001$]. Post-hoc analyses found that as hypothesized, increased length of access to a running wheel results in greater running volume (12 h > 3 h > 1 h at all time points; $p < .05$ for all). In 1 h exercise mice, there was no significant variation in running throughout the course of the experiment; however, in 3 h and 12 h exercise mice, running dropped off in later months compared to early months of intervention.

3.1.2. Speed (rotations/min)

Running speed (rotations/min) was calculated by averaging the number of rotations performed in one-minute bins that were not counted as a “break”, essentially averaging the number of rotations performed per minute when the number of rotations performed in that bin was greater than five. A two way repeated measures ANOVA was performed to determine the effect of treatment on running speed (rotations/min) during each month of the intervention (Fig. 2B). There was a significant main effect of treatment [$F(2, 27) = 8.020$, $p = .002$], with post-hoc analyses showing that 3 h and 12 h mice ran faster than 1 h mice ($p < .001$ for both). These results suggest that mice with longer access to a running wheel actually run faster, possibly due to increased fitness levels, rather than shorter access mice running faster to maximize exercise output. The main effect of time was also significant [$F(7,189) = 41.747$, $p < .001$], with a trend of mice running slower (reduced rotations/min) as time went on during the experiment.

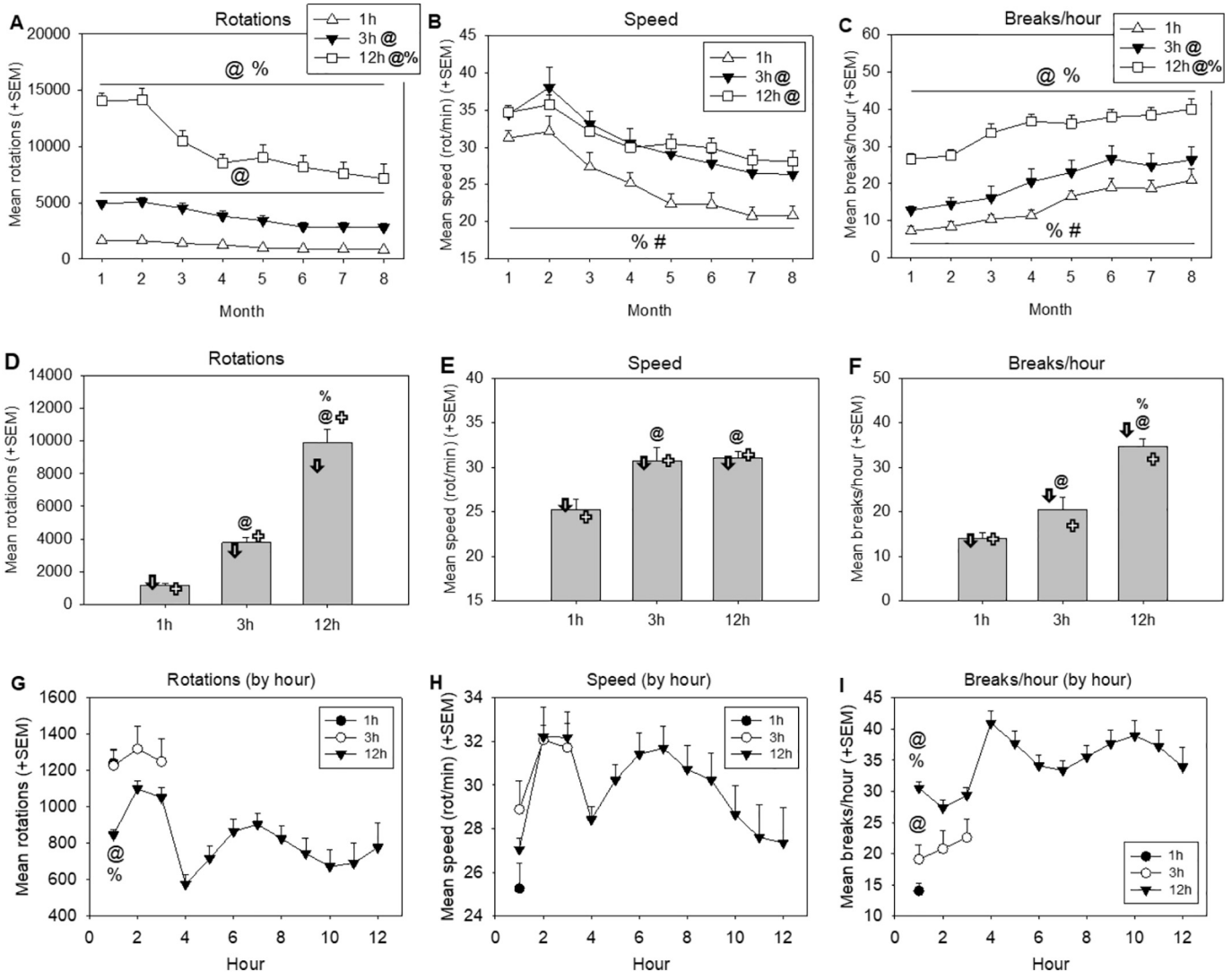

Fig. 2. Graphs represent means (+SEM) of running parameters. (A) The number of rotations performed by each mouse was recorded with computer software in one-minute bins during each exercise session. The total number of rotations performed was summed during each session as a measure of running volume. At all time points, there was a dose-dependent effect (12 h > 3 h > 1 h), and mice ran less as time went on during the experiment. (B) Running speed (rotations/min) was calculated by averaging the number of rotations performed in one-minute bins that were not counted as a "break", essentially averaging the number of rotations performed per minute when the number of rotations performed in that bin was greater than five. Overall, 3 h and 12 h mice ran faster than 1 h mice, and running speed declined in all groups over the course of the experiment. (C) Breaks/h was calculated to determine the number of one minute bins per hour during which the mouse was not running. A "break" was a one-minute bin in which the animal performed less than five wheel rotations. Overall, there was a dose-dependent effect (12 h > 3 h > 1 h), and mice took more breaks as time went on during the experiment. (D) Mean rotations performed throughout the daily wheel access period, averaged over the entire exercise intervention period. An analysis of only the first hour of running found that 12 h mice performed fewer rotations than 1 h and 3 h mice during that period ($p < .05$ for both). (E) Mean running speed throughout the daily wheel access period, averaged over entire exercise intervention period. An analysis of only the first hour of running found no significant difference in running speed during that time period, regardless of exercise group ($p > .05$). (F) Mean breaks/h taken throughout the daily wheel access period, averaged over entire exercise intervention period. An analysis of only the first hour of running found that mice with increased length of access to a running wheel took more breaks (12 h > 3 h > 1 h) during that time period. *@p < .05 versus 1 h; %p < .05 versus 3 h; #p < .05 versus 12 h.* In D–F: arrow = male average, plus sign = female average.

The treatment × time interaction was not significant [$F(14,189) = 1.164$, $p = .306$].

3.1.3. Breaks/h

Breaks/h was calculated to determine the number of one-minute bins per hour during which the mouse was not running. A "break" was a one-minute bin in which the animal performed less than five wheel rotations. A two way repeated measures ANOVA was performed to determine the effect of treatment on breaks/h during each month of the intervention (Fig. 2C). There was a significant main effect of treatment [$F(2, 27) = 29.114$, $p < .001$], with post-hoc analyses showing that mice with shorter access to a running wheel took fewer breaks (12 h > 3 h > 1 h; $p < .05$ for all), supporting the hypothesis that mice with shorter access to a running wheel may take fewer breaks to maximize exercise output. The main effect of time was also significant [$F(7,189) = 33.519$, $p < .001$], with a trend of mice taking more breaks/h as time went on during the experiment. The treatment × time interaction was not significant [$F(14,189) = 0.779$, $p = .691$].

3.1.4. Hourly analysis of running

Running parameters as discussed above were averaged over the entire daily access period (1 h, 3 h, or 12 h). It is possible that running patterns varied over the course of the day, particularly in the first hour or few hours of extended wheel access. Therefore, additional analyses

were run to assess running patterns throughout the duration of daily wheel access. Hourly rotations (Fig. 2D), speed (Fig. 2E), and breaks/h (Fig. 2F) were averaged across the 8 month exercise intervention period.

Mice in the 1 h group performed on average 1241 rotations in the hour of wheel access, while the 3 h and 12 h groups hourly ranges were 1226–1319 and 575–1100 rotations, respectively, suggesting consistent running in the 3 h group but variable running throughout the day in the 12 h group. Mice in the 1 h group ran at a speed of 25.3 rotations/min, while the 3 h and 12 h groups hourly ranges were 28.9–32.1 and 27.0–32.2 rotations/min, respectively, suggesting that 3 h and 12 h mice consistently ran faster than 1 h mice. Mice in the 1 h group took 14.0 breaks in that hour on average, while the 3 h and 12 h groups hourly ranges were 19.1–22.6 and 27.4–40.9 breaks/h, respectively, suggesting a consistent dose-dependent effect of exercise for breaks taken and considerable hourly variability in the 12 h group.

One way ANOVAs were performed to compare running parameters during the first hour of wheel access for all three exercise groups. The main effect of treatment was significant for rotations [$F(2, 27) = 10.735$, $p < .001$] and breaks/h [$F(2, 27) = 32.739$, $p < .001$]; however, running speed did not significantly differ between groups [$F(2, 27) = 1.980$, $p = .158$]. Posthoc analyses found that 12 h mice performed fewer rotations than 1 h and 3 h mice during the first hour of exercise ($p < .05$ for both). Additionally, mice with longer access to a running wheel took more breaks per hour during the first hour of running (12 h > 3 h > 1 h; $p < .05$ for all).

3.2. Physiological measures

3.2.1. Body weight and food intake

A two way repeated measures ANOVA was performed to determine the effect of exercise on body weight over the course of the experiment (Fig. 3A). The main effect of treatment was not significant [$F(3, 36) = 1.786$, $p = .167$]. The main effect of time was significant [$F(8,288) = 194.527$, $p < .001$], and post-hoc analyses found that weight increased during each week of treatment ($p < .05$ for all), except between weeks 4 and 5 ($p > .05$), which likely reflects normal weight gain during the aging process. The treatment × time interaction was significant as well [$F(24,288) = 3.956$, $p < .001$]. Post-hoc analyses found no baseline differences between groups during the pre-intervention period, or in early treatment months (months 1–5; $p > .05$ for all). All exercise groups showed similarly reduced body weight despite sizable differences in running volume and quality. Additionally, these reductions in body weight took several months to become apparent (months 6–8; $p < .05$ for all). Similarly, a one way ANOVA found a significant main effect of treatment [$F(3, 36) = 6.72$, $p < .001$] for weight gain over the course of the experiment (Fig. 3B), such that exercise, regardless of length of access to the running wheel, similarly attenuated weight gain compared to sedentary mice ($p < .05$ for all).

Food intake was normalized to body weight, resulting in the measure of grams of food intake per kilogram of body weight (g/kg). A two way ANOVA was performed to assess the effect of exercise on daily food intake over the course of the experiment (Fig. 3C). The main effect of treatment was significant [$F(3, 36) = 12.576$, $p < .001$], and post-hoc analyses found that there was a dose-dependent increase in food intake with increasing access to a running wheel ($p < .05$ for all), except that 1 h and 3 h mice ate similar amounts (12 h > 3 h = 1 h > sedentary). The main effect of time was also significant [$F(8,277) = 89.672$, $p < .001$], and post-hoc analyses found several significant differences between time points during the experiment. Food intake increased during the first two months of intervention compared to the pre-intervention period ($p < .001$ for both). Food intake then decreased in month 3 ($p < .05$) and remained fairly stable between months 3–7 ($p > .05$ for all), then dropped again during month 8 of intervention ($p < .05$). The treatment × time interaction was significant as well [$F(24,277) = 4.693$, $p < .001$]. Post-hoc analyses found no baseline differences between groups during the pre-intervention period ($p > .05$ for all). Mice in all exercise groups ate more than sedentary mice during most months of intervention ($p < .05$ for all months except month 1 $p = .092$). Mice in the 12 h exercise group ate > 1 h mice in intervention months 1–3, 5–6, and > 3 h mice during all months of exercise intervention ($p < .05$ for all). Mice in the 1 h and 3 h groups ate similarly throughout the entire intervention period ($p > .05$ for all)

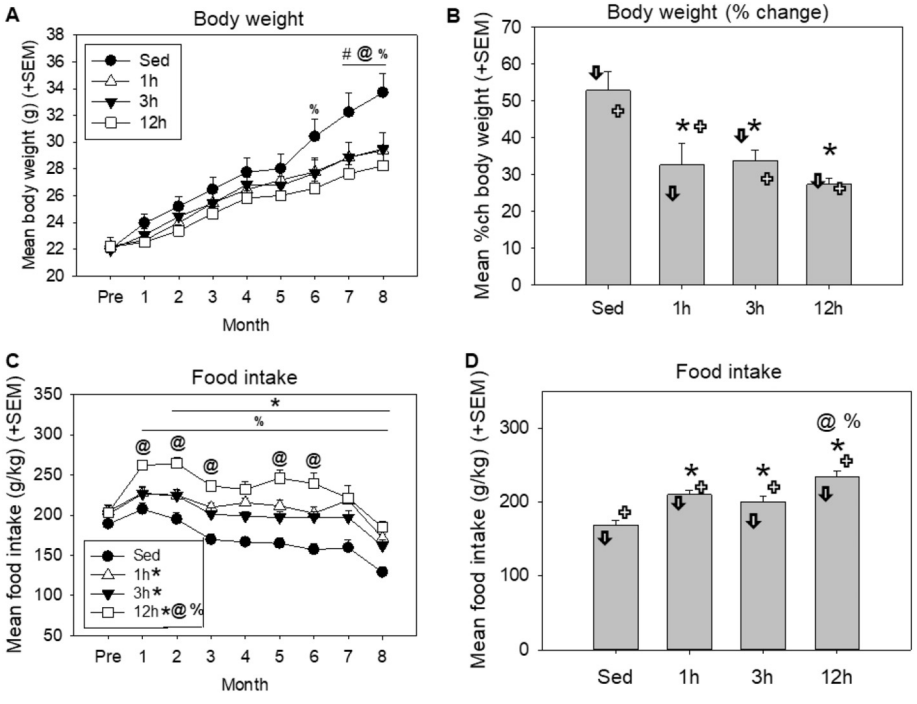

Fig. 3. Mean (+SEM) body weight and food intake over the course of the exercise intervention period. (A) There were no baseline differences in body weight between groups during the pre-intervention period, or in treatment months 1–5. Mice in all exercise groups weighed less than sedentary mice in intervention months 6–8. (B) Exercise, regardless of length of access to the running wheel, similarly attenuated weight gain compared to sedentary mice. (C) Food intake was normalized to body weight, resulting in the measure of grams of food intake per kilogram of body weight (g/kg). Overall, there was a dose-dependent increase in food intake with increasing access to a running wheel, except that 1 h and 3 h mice ate similar amounts (12 h > 3 h = 1 h > sedentary). There were no baseline differences between groups during the pre-intervention period. Mice in all exercise groups ate more than sedentary mice in intervention months 2–8, and this trend was also apparent in month 1. Mice in the 12 h exercise group ate > 1 h mice in intervention months 1–3, 5–6, and > 3 h mice during all months of exercise intervention. Mice in the 1 h and 3 h groups ate similarly throughout the entire intervention period. (D) Food intake was averaged over the course of the intervention period, and normalized to body weight, resulting in the measure of grams of food intake per kilogram of body weight (g/kg). All exercise groups showed increased food intake, with 1 h and 3 h mice eating similarly, and 12 h mice eating the most. *$p < .05$ versus sedentary, @$p < .05$ versus 1 h; %$p < .05$ versus 3 h, #$p < .05$ versus 12 h. In B & D: arrow = male average, plus sign = female average.

despite notable differences in running volume and intensity measures. Similarly, a one way ANOVA found a significant main effect of treatment [F(3, 36) = 15.222, $p < .001$] for food intake averaged over the entire intervention period (Fig. 3D), such that exercise groups showed increased food intake, with 1 h and 3 h mice eating similarly, and 12 h mice eating the most (12 h > 3 h = 1 h > Sed; $p < .05$ for all).

Since there appeared to be dose-dependent effects of exercise on body weight and food intake, correlations were run between these measures and running parameters. While there was no relationship between body weight or food intake with running speed or breaks per hour, there were significant correlations for number of rotations performed with both % body weight change [r(38) = −0.446, $p = .004$] and food intake averaged over the experiment [r(38) = 0.686, $p < .001$]. These results suggest that greater running volume (but not intensity) was associated with reduced body weight, as well as increased food intake, likely in an attempt to compensate for energy deficits under exercise conditions.

3.2.2. Organ and muscle weights

For each organ and muscle collected, a separate one way ANOVA was performed to assess the effect of treatment on raw and normalized (% of body mass) weights of the brain, heart, lungs, liver, kidneys, spleen, pancreas, adrenal glands, soleus, gastrocnemius, and quadricep. Mean and SEM for raw and normalized organ and muscle weights can be seen in Table 1.

A one way ANOVA found a significant main effect of treatment for raw brain mass [F(3, 35) = 3.369, $p = .029$], such that 1 h mice had larger brains than sedentary and 3 h mice ($p < .05$ for both). A one way ANOVA found that treatment did not have a significant effect on normalized brain mass [F(3, 35) = 2.154, $p = .111$].

Although a one way ANOVA found no significant effect of treatment on raw heart mass [F(3, 35) = 1.213, $p = .319$], a one way ANOVA did find a significant main effect of treatment on normalized heart mass [F(3, 35) = 4.634, $p = .008$]. Post-hoc analyses found that 1 h and 12 h mice had bigger hearts (normalized to body weight) compared to sedentary and 3 h mice ($p < .05$ for all).

One way ANOVAs found that treatment did not have a significant main effect on raw [F(3, 36) = 1.334, $p = .278$] or normalized [F(3, 36) = 1.661, $p = .193$] lung mass.

One way ANOVAs found that treatment did not have a significant main effect on raw [F(3, 33) = 0.531, $p = .664$] or normalized [F(3, 33) = 0.603, $p = .618$] liver mass.

Although a one way ANOVA found no significant effect of treatment on raw kidney mass [F(3, 36) = 1.372, $p = .267$], a one way ANOVA did find a significant main effect of treatment on normalized kidney mass [F(3, 36) = 4.822, $p = .006$]. Post-hoc analyses found that 1 h mice had bigger kidneys (normalized to body weight) compared to all other groups ($p < .05$ for all).

One way ANOVAs found that treatment did not have a significant main effect on raw [F(3, 34) = 0.262, $p = .852$] or normalized [F(3, 34) = 0.215, $p = .885$] spleen mass.

A one way ANOVA found that treatment had a significant main effect on raw pancreas mass [F(3, 33) = 3.444, $p = .028$]. Post-hoc analyses found that all exercise groups had a smaller pancreas compared to the sedentary group ($p < .05$ for all except 3 h $p = .075$). When pancreas mass was normalized to body weight, the main effect of treatment approached significance [F(3, 33) = 2.828, $p = .054$]. Post-hoc analyses found that 1 h and 12 h mice had a smaller pancreas (normalized to body weight) than sedentary mice ($p < .05$ for both).

One way ANOVAs found that treatment did not have a significant main effect on raw [F(3, 34) = 0.303, $p = .823$] or normalized [F(3, 34) = 0.102, $p = .959$] adrenal mass.

Although a one way ANOVA found no significant effect of treatment on raw soleus mass [F(3, 33) = 1.128, $p = .352$], a one way ANOVA did find a significant main effect of treatment on normalized soleus mass [F(3, 33) = 4.082, $p = .014$]. Post-hoc analyses found that 3 h and 12 h mice had bigger soleus (normalized to body weight) compared to sedentary mice ($p < .05$ for all).

Table 1
Mean and SEM of raw (g) and normalized (% of body weight) organ and muscle weights for each treatment group.

	Sedentary		1 h		3 h		12 h	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Organ								
Brain mass (g)	0.463	0.005	***0.482***$^{*,\%}$	***0.005***	0.469	0.004	0.473	0.003
Brain mass (%)	1.418	0.067	1.594	0.056	1.500	0.064	1.614	0.063
Heart mass (g)	0.143	0.006	0.158	0.011	0.139	0.006	0.147	0.007
Heart mass (%)	0.434	0.015	***0.506***$^{*,\%}$	***0.025***	0.445	0.009	***0.496***$^{*,\%}$	***0.015***
Lung mass (g)	0.283	0.020	0.246	0.016	0.238	0.010	0.290	0.036
Lung mass (%)	0.843	0.068	0.796	0.048	0.774	0.052	0.983	0.108
Liver mass (g)	1.512	0.076	1.407	0.070	1.477	0.047	1.411	0.085
Liver mass (%)	4.427	0.154	4.660	0.218	4.737	0.116	4.708	0.225
Spleen mass (g)	0.089	0.014	0.082	0.010	0.077	0.005	0.082	0.007
Spleen mass (%)	0.259	0.036	0.278	0.039	0.254	0.025	0.286	0.034
Kidney mass (g)	0.426	0.024	0.485	0.035	0.427	0.020	0.420	0.023
Kidney mass (%)	1.285	0.059	***1.579***$^{*,\%,\#}$	***0.083***	1.360	0.026	1.410	0.044
Adrenal mass (g)	0.0090	0.0010	0.0078	0.0009	0.0086	0.0009	0.0081	0.0010
Adrenal mass (%)	0.027	0.003	0.026	0.003	0.028	0.004	0.029	0.004
Pancreas mass (g)	0.903	0.066	***0.629****	***0.062***	0.733	0.061	***0.651****	***0.066***
Pancreas mass (%)	2.617	0.113	***2.065****	***0.171***	2.320	0.113	***2.156****	***0.144***
Muscle								
Soleus mass (g)	0.0084	0.0004	0.0084	0.0007	0.0094	0.0005	0.0095	0.0005
Soleus mass (%)	0.025	0.001	0.028	0.002	***0.030****	***0.001***	***0.031****	***0.001***
Gastrocnemius mass (g)	0.140	0.007	0.140	0.006	0.140	0.005	0.141	0.005
Gastrocnemius mass (%)	0.410	0.021	***0.464****	***0.014***	***0.450****	***0.006***	***0.476****	***0.007***
Quadricep mass (g)	0.163	0.008	0.165	0.012	0.173	0.009	0.169	0.010
Quadricep mass (%)	0.481	0.028	0.553	0.047	0.548	0.018	0.563	0.026

Values that vary significantly from one or more other groups are bolded and italicized.

* $p < .05$ *versus sedentary.*

$^{\%}$ $p < .05$ *versus 3 h.*

$^{\#}$ $p < .05$ *versus 12 h.*

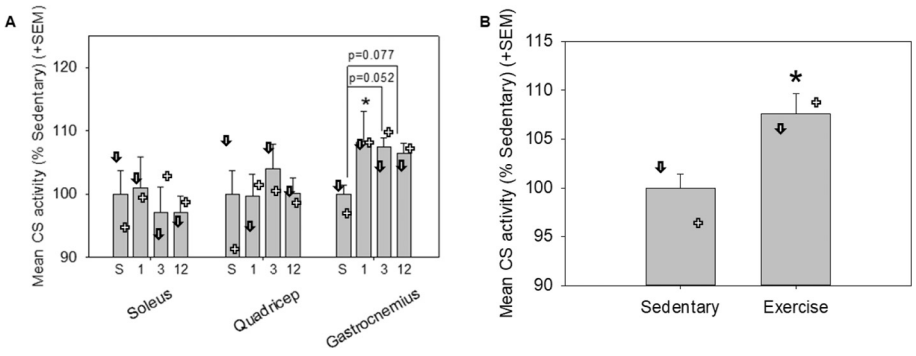

Fig. 4. Mean (+SEM) citrate synthase activity in muscles, represented as percent of mean sedentary value. (A) Citrate synthase activity was higher in the gastrocnemius of 1 h mice compared to sedentary mice (*p < .05). Additionally, the increase in citrate synthase activity in 3 h (p = .052) and 12 h (p = .077) *versus* sedentary mice approached significance. (B) Collapsing the three exercise groups into a single group and comparing it to the sedentary group using a t-test resulted in a significant effect, with exercise mice having greater citrate synthase activity in the gastrocnemius compared to sedentary mice (*p < .05). On x-axis, S = sedentary, 1 = 1 h, 3 = 3 h, 12 = 12 h. Arrow = male average, plus sign = female average.

Although a one way ANOVA found no significant effect of treatment on raw gastrocnemius mass [F(3, 32) = 0.012, p = .998], a one way ANOVA did find a significant main effect of treatment on normalized gastrocnemius mass [F(3, 32) = 4.877, p = .007]. Post-hoc analyses found that all exercise groups had bigger gastrocnemius (normalized to body weight) compared to sedentary mice (p < .05 for all).

One way ANOVAs found that exercise did not have a significant main treatment on raw [F(3, 32) = 0.223, p = .880] or normalized [F(3, 32) = 1.400, p = .261] quadricep mass.

3.2.3. Citrate synthase assays

A one way ANOVA found that the main effect of exercise was not significant for citrate synthase activity in the soleus [F(3, 24) = 0.233, p = .873], quadricep [F(3, 25) = 0.342, p = .795], or gastrocnemius [F(3, 26) = 2.208, p = .111] (Fig. 4A). Post-hoc analyses found that citrate synthase activity was higher in the gastrocnemius of 1 h mice compared to sedentary mice (p = .032). Additionally, the increase in citrate synthase activity in 3 h (p = .052) and 12 h (p = .077) *versus* sedentary mice approached significance. These results suggest that exercise similarly increased citrate synthase in gastrocnemius muscle across exercise conditions despite differences in running volume and intensity measures. Collapsing the three exercise groups into a single group and comparing it to the sedentary group using a *t*-test resulted in a significant effect, t(23) = 2.450, p = .022, with exercise mice having greater citrate synthase activity in the gastrocnemius compared to sedentary mice (Fig. 4B).

3.2.4. ELISA for serum corticosterone concentration

A one way ANOVA found that treatment had no significant effect on resting serum corticosterone levels [F(3, 36) = 1.125, p = .352] (Fig. 5A). Since there appeared to be a trend of all exercise groups showing reduced corticosterone levels compared to the sedentary group, the three exercise groups were collapsed and a *t*-test was used to compare this collapsed exercise group to the sedentary group (Fig. 5B). This difference approached significance [t(38) = 1.739, p = .09], such that there was a trend of exercise mice having reduced corticosterone compared to sedentary mice. Additionally, since this trend of exercise reducing corticosterone levels appeared that it could be dose-dependent, a correlation between corticosterone levels and running parameters were performed. Although the relationship between rotations performed and corticosterone was indeed negative, it was not significant [r(38) = −0.171, p = .292]. Corticosterone's relationships with running speed and breaks/h were also not significant (p > .05). Taken together, these findings suggest that exercise may marginally reduce serum corticosterone levels, with little variation due to differences in volume or intensity.

3.3. Non-cognitive behavioral performance

3.3.1. Rotarod

A one way ANOVA found a significant main effect of treatment on rotarod performance, as measured by amount of time on rod [F(3, 36) = 3.379, p = .029] (Fig. 6A). Post-hoc analyses found that all exercise groups spent a greater amount of time on the rotarod compared to the sedentary group (p < .05 for all). These findings suggest that exercise improves motor function, and that this effect does not appear to be dose-dependent.

3.3.2. Open field

One way ANOVAs found that treatment did not have a significant main effect on distance traveled [F(3, 36) = 1.248, p = .307] in the open field (Fig. 6B). Since there appeared to be a trend of all exercise groups showing increased distance traveled in the open field compared to the sedentary group, the three exercise groups were collapsed and a *t*-test was used to compare this collapsed exercise group to the sedentary group. This difference approached significance [t(38) = 1.731, p = .09], such that there was a trend of exercise traveling a greater distance compared to sedentary mice (data not shown).

A one way ANOVA found that treatment had a significant main effect on center time [F(3, 36) = 4.075, p = .014], and post-hoc analyses found that for both measures, all exercise groups displayed greater center activity compared to the sedentary group (p < .05 for all) (Fig. 7A). Taken together, these results suggest that the effects of exercise on general locomotion and anxiety-like behavior in the open field are significant but do not appear to be dose-dependent.

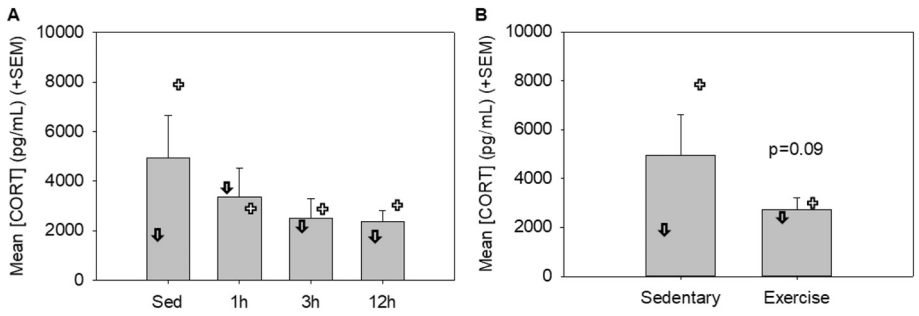

Fig. 5. Mean (+SEM) resting serum corticosterone levels. (A) Treatment had no significant effect on serum corticosterone levels. (B) Since there appeared to be a trend of all exercise groups showing reduced corticosterone levels compared to the sedentary group, the three exercise groups were collapsed and a *t*-test was used to compare this collapsed exercise group to the sedentary group. This difference approached significance (p = .09), such that there was a trend of exercise mice having reduced corticosterone compared to sedentary mice. Arrow = male average, plus sign = female average.

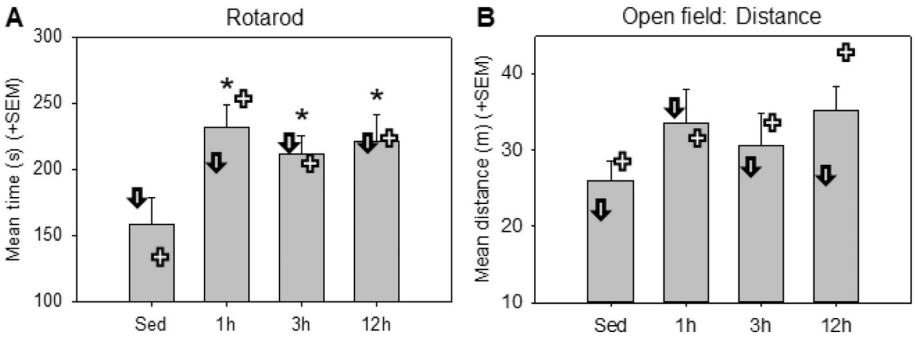

Fig. 6. Performance measures on behavioral tasks assessing motor function. (A) Rotarod performance, as measured by mean (+SEM) time on rod. All exercise groups spent a greater amount of time on the rotarod compared to the sedentary group (*p < .05). (B) Mean (+SEM) distance traveled in a ten-minute open field test. Treatment had no significant effect on this measure of activity. Arrow = male average, plus sign = female average.

3.3.3. Social interaction

A two way repeated measures ANOVA was performed to determine the effect of treatment on time spent sniffing the empty cup *vs.* conspecific cup during the social interaction task (Fig. 7B). According to the creator of this social interaction paradigm, groups can be assessed for exhibiting sociability (preference for conspecific cup *vs.* empty cup) using a repeated measures ANOVA when there are multiple groups, but degree of preference should not be compared as a graded parameter between groups [40]. There was a significant main effect of cup [$F(1, 36) = 19.496$, $p < .001$], such that mice spent more time with the conspecific cup than with the empty cup ($p < .001$). Although the treatment × cup interaction was not significant [$F(3, 36) = 0.678$, $p = .571$], only the 1 h and 12 h mice showed significant preference for the conspecific cup ($p < .05$ for both). Although the trend for sedentary ($p = .135$) and 3 h ($p = .138$) mice were in the same direction (more time spent with conspecific cup than empty cup), these differences did not reach significance, suggesting that exercise may dose-dependently facilitate social behavior.

3.3.4. Marble burying

One way ANOVAs found that treatment did not have a significant main effect on number of digging episodes [$F(3, 36) = 1.072$, $p = .373$] (Fig. 7C), or digging time [$F(3, 36) = 0.650$, $p = .588$] (Fig. 7D). Since there appeared to be trends of exercise dose-dependently affecting digging time, correlations were run between this measure and running parameters. Digging time was correlated with rotations performed [$r(38) = 0.301$, $p = .063$]; however, this only approached significance (data not shown).

3.4. Cognitive behavioral performance

3.4.1. Novel object recognition task

A one way ANOVA found no significant main effect of treatment on the total time of exploration of the two objects during the novel object recognition task [$F(3, 36) = 1.301$, $p = .289$] (Fig. 8A). A two way repeated measures ANOVA was performed to determine the effects of treatment on time spent with the familiar *vs.* novel objects (Fig. 8B). This ANOVA found a significant main effect of object [$F(1, 36) = 89.246$, $p < .001$], and post-hoc testing found that mice spent more time interacting with the novel object than the familiar object ($p < .001$), suggesting intact object recognition memory. The main effect of treatment [$F(3, 36) = 1.301$, $p = .289$] and the object × treatment interaction [$F(3, 36) = 0.929$, $p = .437$] were not significant. However, although there were no differences in exploration of the familiar objects between groups ($p > .05$ for both), 1 h and 12 h exercise mice spent more time interacting with the novel object compared to sedentary mice ($p < .05$ for both), suggesting that exercise may dose-dependently facilitate performance on the novel object recognition task.

3.4.2. Y-maze

One way ANOVAs found no significant main effect of treatment on

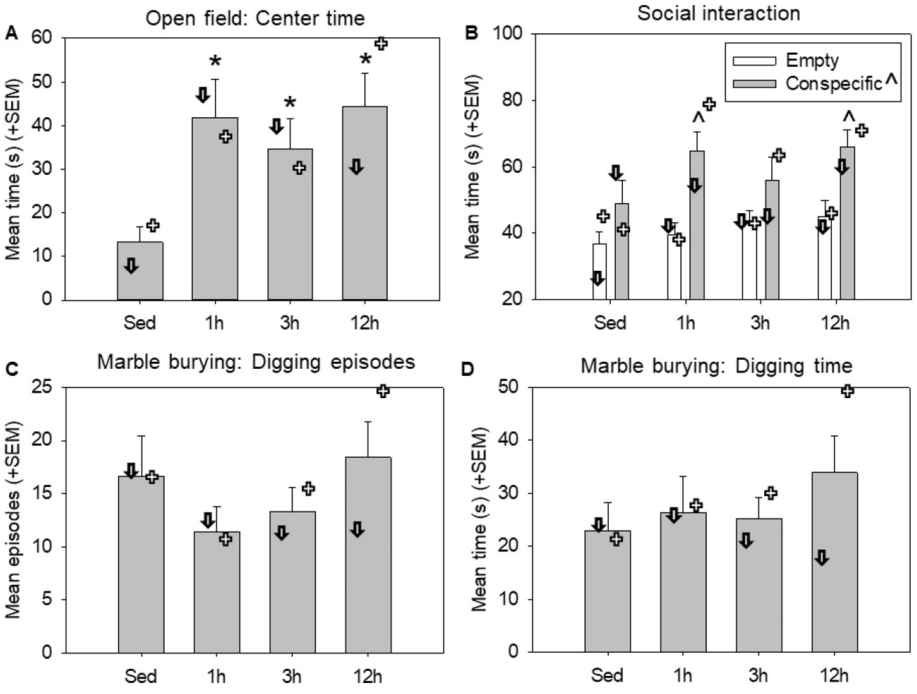

Fig. 7. Performance measures on behavioral tasks assessing temperament and social behavior. (A) Mean (+SEM) center activity during a ten-minute open field test. All exercise groups displayed greater center activity, as measured by time spent in the center of the arena, compared to the sedentary group (*p < .05). (B) Mean (+SEM) time spent with the empty and conspecific mouse-containing cups in the social interaction test. There was a significant main effect of cup, such that mice spent more time with the conspecific cup than with the empty cup (^p < .05); however, only the 1 h and 12 h mice showed significant preference for the conspecific cup (^p < .05 for both). Although the trend for sedentary ($p = .135$) and 3 h ($p = .138$) mice were in the same direction (more time spent with conspecific cup than empty cup), these differences did not reach significance. (C–D) Mean (+SEM) measures of performance on the marble burying task. Treatment had no significant effect on (C) number of digging episodes, or (D) time spent digging. Arrow = male average, plus sign = female average.

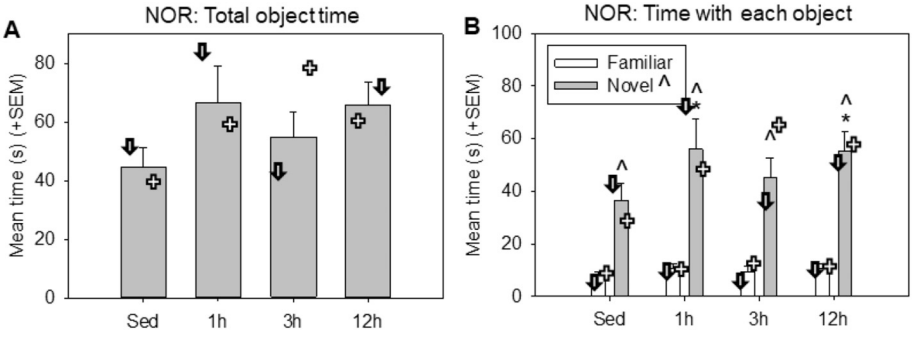

Fig. 8. Mean (+SEM) measures of performance on the novel object recognition (NOR) task. (A) There was no significant treatment effect on total time spent exploring objects ($p > .05$). (B) There was a main effect of object, such that mice spent more time interacting with the novel object than the familiar object, and this was significant for all groups (^$p < .05$); however, although there were no differences in exploration of the familiar objects between groups ($p > .05$ for both), 1 h and 12 h exercise mice spent more time interacting with the novel object compared to sedentary mice (*$p < .05$ for both). Arrow = male average, plus sign = female average.

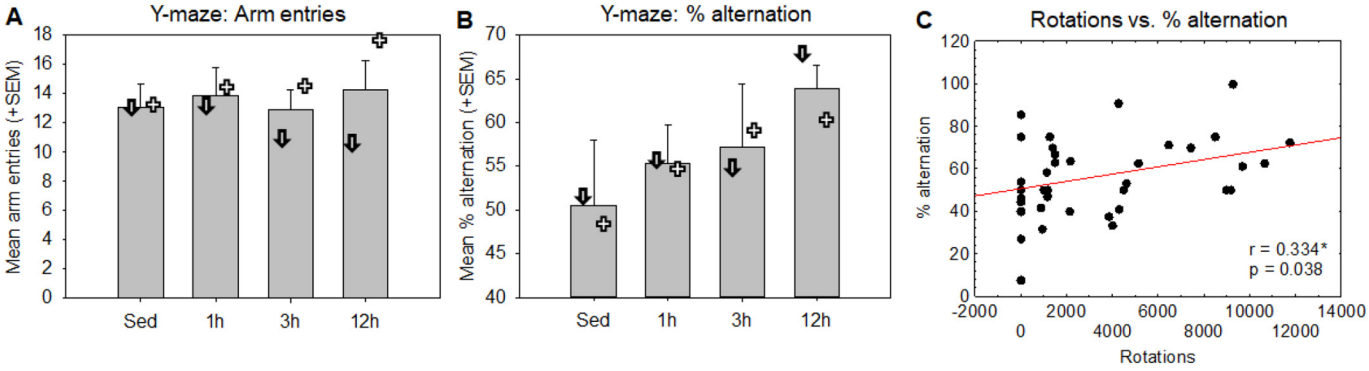

Fig. 9. (A–B) Mean (+SEM) measures of performance on the Y-maze for spontaneous alternation. There were no significant treatment differences for (A) number of arm entries, or (B) % alternation. (C) Rotations performed significantly correlated with % alternation [$r(38) = 0.334$, $p = .038$], suggesting that a greater running volume was associated with enhanced performance on this task assessing spatial working memory (*$p < .05$). Arrow = male average, plus sign = female average.

arm entries [$F(3, 36) = 0.144$, $p = .933$] (Fig. 9A) or % alternation [$F(3, 36) = 0.920$, $p = .441$] (Fig. 9B) in the Y-maze. Since there appeared to be a trend of exercise dose-dependently affecting % alternation, correlations were run between this measure and running parameters. Only rotations performed significantly correlated with % alternation [$r(38) = 0.334$, $p = .038$] (Fig. 9C), suggesting that a greater running volume was associated with enhanced performance on this task assessing spatial working memory.

3.4.3. Barnes maze

3.4.3.1. Latency to find. A two way repeated measures ANOVA was performed to assess the effect of treatment across time (days) on latency to find the escape hole in the Barnes maze task (Fig. 10A). This ANOVA found a significant main effect of time [$F(4,144) = 6.852$, $p < .001$], such that mice were faster to find the escape hole on days 4 and 5 compared to days 1–3 ($p < .05$ for all). The main effect of treatment approached significance [$F(3, 36) = 2.591$, $p = .068$], and post-hoc analyses found that 3 h mice took longer to find the escape box compared to sedentary mice ($p = .010$). The treatment × time interaction was not significant [$F(12,144) = 0.744$, $p = .707$].

A one way ANOVA was performed to determine the effects of treatment on latency to find the escape hole in the Barnes maze, averaged across days 2–5 (Fig. 10B). This ANOVA found a marginally significant main effect of exercise [$F(3, 36) = 2.872$, $p = .050$], and post-hoc analyses found that 3 h mice took longer to find the escape box compared to sedentary mice ($p = .010$).

3.4.3.2. Hole entry rate. Since there appeared to be group differences in latency to find the escape box, hole entry rate was assessed, which is the average amount of time (s) between subsequent hole entries, to determine if this difference could be due to slower rates of exploration. A two way repeated measures ANOVA was performed to assess the effect of treatment across time (days) on hole entry rate in the Barnes maze task (Fig. 10C). This ANOVA found a significant main effect of time [$F(4,144) = 7.682$, $p < .001$], such that mice were faster to travel from one hole to the next as testing days went on. The main effect of treatment was also significant [$F(3, 36) = 3.810$, $p = .018$], such that 3 h mice took longer to travel from hole to hole compared to all other groups ($p < .05$ for all except 12 h $p = .083$), in line with the 3 h group having a longer latency to find the escape hole. The treatment × time interaction was not significant [$F(12,144) = 1.162$, $p = .316$].

A one way ANOVA was performed to determine the effects of treatment on hole entry rate in the Barnes maze, averaged across days 2–5 (Fig. 10D). This ANOVA found a significant main effect of treatment [$F(3, 36) = 4.312$, $p = .011$], with trends of exercise mice traversing more slowly from hole to hole than sedentary mice. Post-hoc analyses found that 3 h mice were slower traveling between holes compared to all other groups ($p < .05$ for all except 12 h $p = .073$). The 12 h mice also moved from hole to hole more slowly than sedentary mice, though this only approached significance ($p = .092$). These findings suggest that longer access to a running wheel may slow exploration speed.

3.4.3.3. Errors and re-entry errors. "Errors" were counted as the number of hole visits before finding the escape box hole. A two way repeated measures ANOVA was performed to assess the effect of treatment across time (days) on errors in the Barnes maze task (Fig. 10E). This ANOVA found a significant main effect of time [$F(4,144) = 7.290$, $p < .001$], such that mice made more errors on day 1 compared to all other days ($p < .05$ for all). The main effect of treatment [$F(3, 36) = 0.712$, $p = .551$] and the treatment × time interaction [$F(12,144) = 1.343$, $p = .201$] were not significant.

A one way ANOVA was performed to determine the effects of treatment on errors in the Barnes maze, averaged across days 2–5 (Fig. 10F). This ANOVA found no significant main effect of exercise [$F(3, 36) = 1.726$, $p = .179$], though post-hoc analyses found that 1 h mice made fewer errors than sedentary mice, though this difference

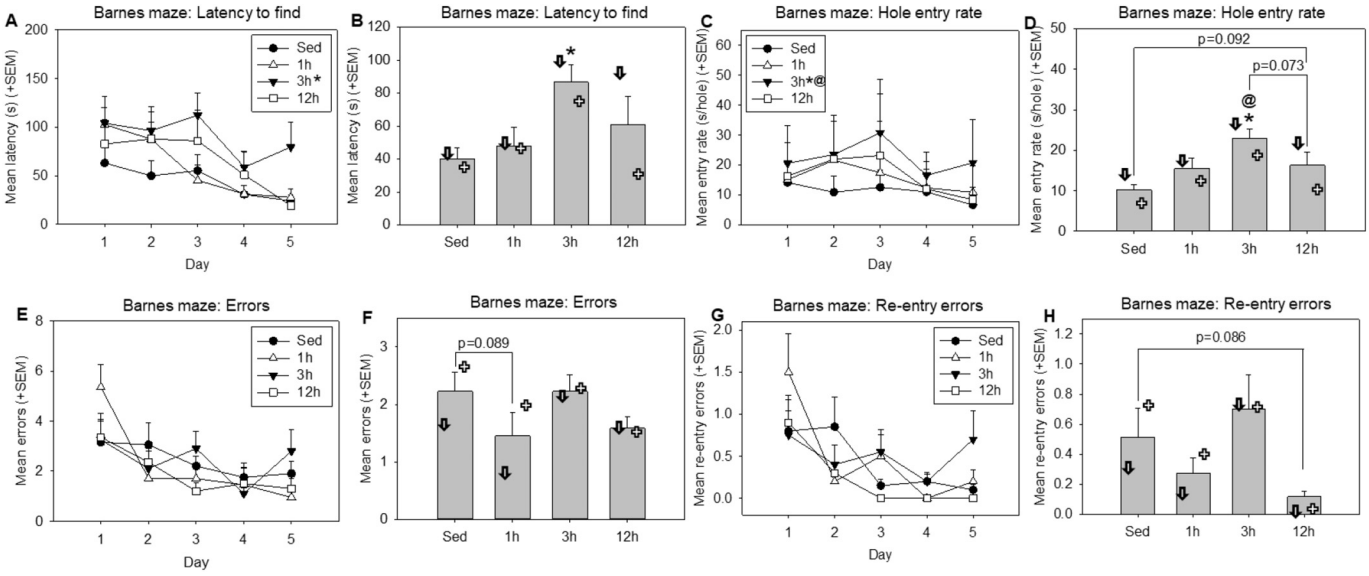

Fig. 10. Mean (+SEM) measures of performance in the Barnes Maze. (A) Latency to find the escape hole across days of the Barnes maze task. Overall, mice exhibited increased performance (shorter latency) by the end of the five days. Mice in the 3 h group had a longer latency compared to all other groups. (B) Average latency to find the escape hole on days 2–5 of the Barnes maze task. Mice in the 3 h group had a longer latency compared to all other groups. (C) Hole entry rate (average time between subsequent hole visits) across days of the Barnes maze task. Overall, mice were faster to travel from one hole to the next as testing days went on, and 3 h mice took longer to travel from hole to hole compared to all other groups ($p < .05$ for all except 12 h $p = .083$), in line with the 3 h group having a longer latency to find the escape hole. (D) Hole entry rate (average time between subsequent hole visits) on days 2–5 of the Barnes maze task. There were trends of exercise mice traversing more slowly from hole to hole than sedentary mice. Specifically, 3 h mice were slower traveling between holes compared to all other groups ($p < .05$ for all except 12 h $p = .073$). The 12 h mice also moved from hole to hole more slowly than sedentary mice, though this only approached significance ($p = .092$). (E) Errors (incorrect hole visits before finding escape hole) across days of the Barnes maze task. Overall, mice exhibited increased performance (fewer errors) by the end of the five days. There was no difference in performance between treatment groups. (F) Average errors on days 2–5 of the Barnes maze task. Mice in the 1 h group made fewer errors than sedentary mice, though this difference only approached significance ($p = .089$). (G) *Re*-entry errors (repeated incorrect hole visits before finding escape hole) across days of the Barnes maze task. Overall, mice exhibited increased performance (fewer errors) by the end of the five days. There was no difference in performance between treatment groups. (H) Average re-entry errors on days 2–5 of the Barnes maze task. Mice in the 12 h group made fewer re-entry errors than sedentary mice, though this difference only approached significance ($p = .086$). *$p < .05$ versus sedentary*, @ $p < .05$ *versus 1 h.* Arrow = male average, plus sign = female average.

only approached significance ($p = .089$).

"*Re*-entry errors" were counted as the number of hole visits repeated to a previously visited hole before finding the escape box hole. A two way repeated measures ANOVA was performed to assess the effect of treatment across time (days) on re-entry errors in the Barnes maze task (Fig. 10G). This ANOVA found a significant main effect of time [$F(4,144) = 8.932$, $p < .001$], such that mice made more errors on day 1 compared to all other days ($p < .05$ for all). The main effect of treatment [$F(3, 36) = 1.460$, $p = .242$] and the treatment × time interaction [$F(12,144) = 1.470$, $p = .142$] were not significant.

A one way ANOVA was performed to determine the effects of treatment on re-entry errors in the Barnes maze, averaged across days 2–5 (Fig. 10H). This ANOVA found that the main effect of exercise approached significance [$F(3, 36) = 2.625$, $p = .065$], and post-hoc analyses found that 12 h mice made fewer re-entry errors than sedentary mice, though this difference only approached significance ($p = .086$). Taken together, these findings suggest that exercise may facilitate spatial learning and memory, as measured by decreases in errors and re-entry errors.

4. Discussion

Although the benefits of cardiovascular exercise (CVE) on healthy aging have been extensively investigated in rodents, most studies have tested a single exercise treatment group (usually unlimited running wheel access), resulting in large volumes of exercise that may not be clinically relevant. In agreement with this, the results of the current study indicate that mice given access to a running wheel for the entirety of their active cycle ran for about 50% of the time, or six hours. Therefore, it was of interest to determine whether smaller "doses" of CVE could exert similar benefits. Additionally, we hypothesized that giving mice different lengths of access to an exercise wheel may result in exercise regimens that varied not only by volume, but by intensity (as defined by several measures, discussed below) as well. Moreover, previous studies have generally utilized a shorter intervention period (ranging from a few weeks to a few months), while there is evidence suggesting that earlier, longer, or even life-long exercise interventions may have different effects [5, 17, 47]. This is the first long-term intervention study examining different daily levels of CVE to model a human life span of varying volume and intensity exercise regimens on physiological measures and a comprehensive behavioral battery assessing motor function, temperament, and cognition.

4.1. Varying access time to running wheels produces differences in running volume and intensity

There has been debate in the literature regarding the efficacy of exercise to produce health benefits regarding volume and intensity. Some studies suggest that longer-duration exercise is more beneficial compared to regimens shorter in duration [5]. Others suggest that only small amounts of exercise are necessary to see significant health benefits [24, 25], and there is evidence that shorter duration high-intensity regimens are equally beneficial to longer-duration/lower-intensity regimens in regard to cardiovascular and metabolic function and musculoskeletal benefits [26–29]. Therefore, creating exercise regimens of different doses with varying duration/volume and intensity was of interest to test their effects on physiology, behavior, and neurobiology. Although studies have previously utilized varying duration and intensity of exercise using forced exercise (*e.g.*, treadmill running), fewer studies have attempted to create different "doses" of exercise using

voluntary wheel running. While treadmill running is a known stressor, some studies have shown that voluntary wheel running is significantly less stressful [33–36]. Additionally, wheel running has the benefit of allowing for correlational analyses of volume and intensity measures with outcome measures.

Most studies that utilize wheel running as the form of exercise provide unlimited wheel access. As nocturnal animals, mice will not usually run during their light (sleep) cycle [48]. Therefore, our 12 h group is likely comparable to the unlimited wheel access groups in other studies. Compared to 12 h mice, 1 h mice performed 88% fewer wheel rotations, ran 19% slower, and took 60% fewer breaks; whereas 3 h mice performed 62% fewer wheel rotations, ran at the same speed (1% slower), and took 40% fewer breaks. There was a dose-dependent increase in running volume, such that, as hypothesized, animals that had longer access to a running wheel performed a greater number of wheel rotations. It was also hypothesized that animals with shorter access to a running wheel may attempt to compensate by increasing running speed or taking fewer breaks to maximize running volume. In agreement with this, we found that 1 h and 3 h mice took about half as many breaks compared to 12 h animals. However, mice with shorter access to a running wheel did not run faster to compensate; instead, 3 h and 12 h mice ran faster than 1 h mice, which may be due to increased fitness levels in these groups or simply represent the physiological limits of mice to sustain nearly continuous exercise for an hour. These findings suggest that providing mice with different lengths of access to a running wheel produces exercise regimens that not only differ in exercise volume, but also measures that may be indicative of some aspect of running intensity. The 1 h group ran the least and at the lowest speed but took the fewest number of breaks. The 3 h group ran an intermediate volume at a higher speed and took and took an intermediate number of breaks. The 12 h group ran the most and at a higher speed and took the most number of breaks.

These findings raise a perhaps more important question about the nature of aerobic exercise "intensity" as a construct, with regard to its equivalency between human studies and those in rodents. The construct of exercise volume (defined as the accumulated amount of exercise per some unit of time such as a day, week or month for humans, and total rotations per session for our mice; Fig. 2A) is unambiguously defined presently and dose-dependent as arrayed across our running wheel access periods. Intensity is a more difficult conceptual challenge, presently. It should first be noted that intensity can be defined differently in various human settings. Clinical studies and from public service sources such as the Centers for Disease Control often refer to exercise intensity for humans in somewhat imprecise terms (such as "light", "moderate" or "vigorous") [49]. At the other extreme, "gold standard" laboratory methods establish intensity referenced to metabolic capacity (*e.g.* VO_2 maximum, heart rate maximum or lactate threshold) using physiological measurement apparatus in controlled conditions that do not transfer readily out of the lab, though reasonably accurate estimations can be made using various formulae [50]. More convenient methods used to define intensity in humans for conditioning purposes are heart rate ranges indexed to age, power (sustained force) and perceived exertion on a numerical rating scale [51–53]. The latter measure is clearly not obtainable from rodents and getting heart rates or lactate thresholds, while possible in rodent engaging in exercise, were not practical measurements to take in a chronic running wheel format [54]. Therefore, we must look to the exercise parameters themselves in the present study to define the construct.

Classic heart rate zone-based training, such as high intensity interval training (HIIT) regimens, define workout intensity usually in terms of periods of exertion at fixed heart rate targets followed by precise, defined rest periods [55]. The running parameters that we measured in the mice do have distinct on/off periods that model this. However, our analyses include several potential measures of intensity based on the periods of time within which one integrates "on" periods with "off" periods. "Speed" (rotations per minute; Fig. 2B), which may mimic short intense bouts of exercise, such as Tabata intervals, is identical and highest for 3 and 12 h groups, and may estimate the maximum exertion they are willing to produce [56]. In contrast, the exercise period frequency or "density" (defined as the number of breaks per hour) reflects a somewhat different definition of intensity, not as clearly translatable to human HIIT routines (Fig. 2C). Furthermore, within-session analyses reveal yet other relevant patterns, in which both 1 h and 3 h groups defend the same high density of running, but in different ways: the 1 h group took fewer breaks whereas the 3 h group maintained a higher speed when they did run. The 12 h animals ran at higher densities earlier in the dark phase, producing a range of intensities. This discussion clearly raises more questions than it can answer, but nonetheless illustrates that future preclinical work that looks at rodent disease models will need to carefully consider these conceptual challenges and may want to anchor these observed patterns acute with laboratory tests in rodents that estimate lactate threshold and other metabolic measures that serve as the basis for human studies.

4.2. Exercise volume-dependently alters physiology

All doses of exercise resulted in similarly reduced body weight gain, which only became apparent during the last 3 months of exercise. Generally, there was a volume-dependent increase in food intake; however, no differences were seen between the 1 h and 3 h groups, despite 3 h mice running nearly three times as much as 1 h mice and exhibiting similar weight gain. There was also a significant positive correlation between running volume and food intake, and a significant negative correlation between running volume and body weight gain. Taken together, these findings suggest that mice will increase food intake when exercising more, likely to compensate for increased energy expenditure to maintain a healthy body weight. Additionally, there was no relationship between food intake or weight gain and running speed or breaks/h, suggesting that these physiological effects may be more likely mediated by exercise volume rather than intensity.

Interestingly, although there were trends in each exercise group, only 1 h mice showed a significant increase in raw brain mass compared to sedentary mice, which cannot be attributed to differences in body mass (since they weighed less than sedentary mice). This increased brain mass may be due, in part, to synaptogenesis, and cell and synapse survival during aging [57, 58]. Brain volume is correlated with cardiovascular fitness in the elderly [59], and an experimental study found that aerobic exercise increased the volume of both gray and white matter regions in the aging population, suggesting a sparing of tissue [60]. Mice in the 1 h group also had larger kidneys (normalized to body mass) compared to all other groups. This is likely not fully attributable to a decrease in body mass with exercise, as exercise similarly attenuated body mass in all exercise groups, but only the kidney mass of 1 h mice appears to be affected. Exercise has been shown to alter renal function [61] and improve renal function in patients with chronic kidney disease [62]. Additionally, exercise (1 h & 12 h groups) also increased the normalized mass of the heart compared to sedentary animals. Previous studies have noted cardiac hypertrophy as an adaptation in response to aerobic exercise [63–65]. Lastly, exercise (1 h & 12 h) reduced the size of the pancreas, and this was true for both raw and normalized masses, in contrast to a previous exercise study [66].

Exercise was also shown to increase relative muscle mass, in agreement with prior findings [66], and this effect was similar across groups. This finding, however, may be attributable to a reduction in fat mass (and overall body weight), rather than an actual increase in muscle, as raw muscle mass was unaffected by exercise. Mitochondrial function in these muscles was assessed using citrate synthase activity assays. Citrate synthase activity has been used as an objective fitness measure, signifying aerobic capacity. Exercise increased citrate synthase activity (relative to protein content) in the gastrocnemius muscle only. The gastrocnemius is composed of red muscle fibers, and is referred to as a "fast-twitch" muscle since it is involved in fast movement

like running and jumping. The soleus (a "slow-twitch" muscle composed of white muscle fibers, involved in standing and walking) and quadricep (a muscle composed of a mix of red and white fibers) were less affected by exercise intervention in the current study. Interestingly, CS activity was similarly increased across all exercise groups, but the increase seen was < 10% above sedentary levels. Previous studies have shown that citrate synthase and other mitochondrial enzyme levels in muscle increase following exercise training in both rodents and humans, with decreases seen following weeks to months of exercise cessation [67–71]. Since exercise training ended about a month before tissue collection, this could explain why increases in citrate synthase were small in the gastrocnemius and non-significant in other muscles, in the current study.

There was a trend of exercise reducing resting serum corticosterone levels, and this effect did not appear to be exercise pattern-dependent. Corticosterone levels were also not significantly correlated with measures of running volume or intensity. In previous studies, intense aerobic exercise has been shown to elevate glucocorticoid release [72], and reductions in resting corticosterone levels may represent adaptations of the hypothalamic-pituitary-adrenal axis in response to chronic exercise.

4.3. Exercise improves motor function without significantly affecting general locomotor activity or exploration

All exercise patterns equally improved motor coordination and balance, as measured by performance on the rotarod task. No pattern of exercise had a significant effect on general activity levels or exploratory behavior as measured by activity in the open field and arm entries in the Y-maze. Previous studies in mice have also found no effects of exercise on general activity levels in the open field [73, 74]. While these findings suggest that exercise improves motor function without significantly affecting general locomotor activity or exploration, future additional tests such as grip strength and balance beam walking could further elucidate specific aspects of motor function that benefit from long-term wheel running.

4.4. Exercise reduces anxiety-like behavior and facilitates social behavior and marble burying

All exercise groups produced similar reductions in anxiety-like behavior in the open field (as displayed by more center activity). These findings are in agreement with previous studies that have found that exercise reduces anxiety-like behavior in animals [73–76] and anxiety measures in humans [2, 77, 78]. Furthermore, these findings indicate that even small doses of exercise are potentially anxiolytic. Group differences were also observed in social behavior on the three-chamber social interaction paradigm. Although there were trends of all mice spending more time with the conspecific cup compared to the empty cup, this was only significant for 1 h and 12 h exercise mice. These findings suggest that exercise may facilitate social behavior, which may be linked to a reduction in anxiety. However, while anxiolytic-like effects of 3 h exercise were seen in the open field task, this benefit was not measurable using the social interaction paradigm, which may be a better indicator of social rather than more general anxiety. Previously, exercise animals that showed reduced anxiety-like behavior in the open field and other tasks also showed increased social behavior [76]. Lastly, although there were no group differences, there were trends of exercise dose-dependently facilitating digging behavior in the marble burying task, in addition to correlations of digging time with both running volume and speed. Burying behavior has also been used as a test of anxiety [79, 80], though performance on burying tasks has been shown not to correlate with performance on other tests of anxiety [79, 81], as seen in the current study. It is also argue that marble burying is more reflective of a repetitive and perseverative behavior [81], and others have shown trends that exercised rodents show increased marble burying compared to sedentary controls [82]. Future additional tests including elevated plus maze or light/dark box for anxiety, and tail suspension or forced swim test for depression, could help further elucidate the effects of various exercise patterns on emotionality.

4.5. Exercise enhances cognitive-measure performance depending on dose

All groups of mice showed a significant preference for interacting with the novel object compared to the familiar object during the test phase of the novel object recognition task, a test of non-spatial memory. Exercise did not significantly affect total object exploration time or time with the familiar object, with exercising mice spending more time with the novel object than sedentary mice. Although this trend was apparent for all exercise groups, it was only significant for the 1 h and 12 h groups. Additionally, although there were no group differences, there were trends of exercise dose-dependently enhancing performance on the y-maze for spontaneous alternation task, a test of spatial working memory. In this task, there was also a significant positive correlation of the % alternation with running volume measures. Moreover, there were trends of 1 h and 12 h mice committing fewer errors and re-entry errors on the Barnes maze, a test of spatial memory, while speed of exploration of the Barnes maze was dose-dependently reduced by exercise, possibly as a result of reduced anxiety (as seen in the open field center time). Taken together, these results suggest that exercise dose-dependently enhances cognitive performance on a number of tasks, in agreement with several previous studies in animals and humans [37, 83–88]. While the lowest (1 h) and highest (12 h) doses appeared to be similarly beneficial for novel object recognition (a non-spatial memory task) and Barnes maze task (a hippocampal-dependent/spatial memory task [89]), Y-maze performance (a prefrontal cortex- and hippocampal-dependent/spatial working memory task [90, 91]) seemed to benefit most from higher volumes of exercise.

5. Conclusions

This study represents the first long-term (8 months) intervention study examining different daily levels of exercise to model a life span of regular aerobic exercise in a human on physiological response measures and a comprehensive behavioral battery assessing motor function, temperament and cognition. Varying length of access to a running wheel resulted in exercise regimens that differed by not only volume, but by measures of intensity as well. Exercise reduced body weight and increased relative muscle mass similarly across groups. While all exercise groups showed increased food intake, this was greatest in the 12 h group but did not differ between 1 h and 3 h mice. Additionally, all exercise groups showed improved motor function on the rotarod, and reduced anxiety in the open field. While exercise dose-dependently increased working memory performance in the y-maze, the 1 h and 12 h groups showed the largest changes in the mass of many organs, as well as alterations in several behaviors including social interaction, novel object recognition, and Barnes maze performance. It is likely that some of the effects of exercise were variable and/or diminished, particularly later behavioral assays and end-point physiological measures, as exercise was discontinued prior to the start of behavior testing. Therefore, authors have included results of analyses that trend towards significance, which may point to areas for future study using a shorter interval between the end of exercise and testing. Additionally, the current study included both males and females; however, separating analyses by sex results in an abundant loss of power. Because sex differences have been reported previously in voluntary running patterns and behavioral responses to running [*e.g.* [45, 46]], symbols were added to figures to allow evaluation of these presently. However, future studies powered to confidently include sex as a biological variable are necessary. Overall, our findings suggest that there are widespread effects of long-term exercise on physiology, behavior, and cognition, and that even relatively small amounts of exercise can provide lasting

benefits in mice.

Conflict of interest statement

The authors have no conflicts of interest to declare.

Acknowledgments

This work was supported by NIH grant AG048935. The authors would like to thank Nikita Francis, Sarah Amrein, Wendi Liu, Regina Kim, Joshua Hatfield, Joseph Sullivan, Judianne Davis, Feng Xu, Ralph Molaro, and Robert Chorley for their technical assistance.

References

[1] Physical Activity Guidelines for Americans, https://health.gov/paguidelines/pdf/paguide.pdf, (2008).
[2] S.J. Petruzzello, D.M. Landers, B.D. Hatfield, K.A. Kubitz, W. Salazar, A meta-analysis on the anxiety-reducing effects of acute and chronic exercise, Sports Med. 11 (3) (1991) 143–182.
[3] P. Salmon, Effects of physical exercise on anxiety, depression, and sensitivity to stress: a unifying theory, Clin. Psychol. Rev. 21 (1) (2001) 33–61.
[4] J. Barton, M. Griffin, J. Pretty, Exercise-, nature-and socially interactive-based initiatives improve mood and self-esteem in the clinical population, Perspect. Public Health 132 (2) (2012) 89–96.
[5] S. Colcombe, A.F. Kramer, Fitness effects on the cognitive function of older adults: a meta-analytic study, Psychol. Sci. 14 (2) (2003) 125–130.
[6] A.F. Kramer, K.I. Erickson, S.J. Colcombe, Exercise, cognition, and the aging brain, J. Appl. Physiol. 101 (4) (2006) 1237–1242.
[7] J.E. Ahlskog, Y.E. Geda, N.R. Graff-Radford, R.C. Petersen (Eds.), Physical exercise as a preventive or disease-modifying treatment of dementia and brain aging, Mayo Clin. Proc. 86 (9) (2011) 876–884 (Elsevier).
[8] K.I. Erickson, M.W. Voss, R.S. Prakash, C. Basak, A. Szabo, L. Chaddock, et al., Exercise training increases size of hippocampus and improves memory, Proc. Natl. Acad. Sci. 108 (7) (2011) 3017–3022.
[9] Z. Radak, N. Hart, L. Sarga, E. Koltai, M. Atalay, H. Ohno, et al., Exercise plays a preventive role against Alzheimer's disease, J. Alzheimers Dis. 20 (3) (2010) 777–783.
[10] N.R. Cook, M.S. Albert, L.F. Berkman, D. Blazer, J.O. Taylor, C.H. Hennekens, Interrelationships of peak expiratory flow rate with physical and cognitive function in the elderly: MacArthur Foundation studies of aging, J. Gerontol. 50A (6) (1995) M317–M323, http://dx.doi.org/10.1093/gerona/50A.6.M317.
[11] D.J. Bunce, A. Barrowclough, I. Morris, The moderating influence of physical fitness on age gradients in vigilance and serial choice responding tasks, Psychol. Aging 11 (4) (1996) 671.
[12] S.I. Offenbach, W.J. Chodzko-Zajko, R.L. Ringel, Relationship between physiological status, cognition, and age in adult men, Bull. Psychon. Soc. 28 (2) (1990) 112–114.
[13] T. Abourezk, T. Toole, Effect of task complexity on the relationship between physical fitness and reaction time in older women, J. Aging Phys. Act. 3 (3) (1995) 251–260.
[14] A.F. Kramer, S. Hahn, E. McAuley, N.J. Cohen, M.T. Banich, C. Harrison, et al., Exercise, Aging and Cognition: Healthy Body, Healthy Mind. Human Factors Interventions for the Health Care of Older Adults, (2001), pp. 91–120.
[15] F. Sofi, D. Valecchi, D. Bacci, R. Abbate, G. Gensini, A. Casini, et al., Physical activity and risk of cognitive decline: a meta-analysis of prospective studies, J. Intern. Med. 269 (1) (2011) 107–117.
[16] J. Kulmala, A. Solomon, I. Kåreholt, T. Ngandu, T. Rantanen, T. Laatikainen, et al., Association between mid-to late life physical fitness and dementia: evidence from the CAIDE study, J. Intern. Med. 276 (3) (2014) 296–307.
[17] K.M. Sink, M.A. Espeland, C.M. Castro, T. Church, R. Cohen, J.A. Dodson, et al., Effect of a 24-month physical activity intervention vs health education on cognitive outcomes in sedentary older adults: the LIFE randomized trial, JAMA 314 (8) (2015) 781–790.
[18] H. Van Praag, G. Kempermann, F.H. Gage, Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus, Nat. Neurosci. 2 (3) (1999) 266–270.
[19] H. Van Praag, G. Kempermann, F.H. Gage, Neural consequences of enviromental enrichment, Nat. Rev. Neurosci. 1 (3) (2000) 191–198.
[20] H. Van Praag, T. Shubert, C. Zhao, F.H. Gage, Exercise enhances learning and hippocampal neurogenesis in aged mice, J. Neurosci. 25 (38) (2005) 8680–8685.
[21] B. Anderson, D. Rapp, D. Baek, D. McCloskey, P. Coburn-Litvak, J. Robinson, Exercise influences spatial learning in the radial arm maze, Physiol. Behav. 70 (5) (2000) 425–429.
[22] S. Vaynman, Z. Ying, F. Gomez-Pinilla, Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition, Eur. J. Neurosci. 20 (10) (2004) 2580–2590.
[23] J. Weuve, J.H. Kang, J.E. Manson, M.M. Breteler, J.H. Ware, F. Grodstein, Physical activity, including walking, and cognitive function in older women, JAMA 292 (12) (2004) 1454–1461.
[24] S. Beddhu, G. Wei, R.L. Marcus, M. Chonchol, T. Greene, Light-intensity physical activities and mortality in the United States general population and CKD subpopulation, Clin. J. Am. Soc. Nephrol. 10 (7) (2015) 1145–1153 (CJN. 08410814).
[25] D. Hupin, F. Roche, M. Garet, V. Gremeaux, J. Barthelemy, M. Oriol, et al., Relation between physical activity and morbi-mortality of elderly people: the proof cohort study, Eur. J. Prev. Cardiol. 22 (2015) S91.
[26] M.J. Gibala, J.P. Little, M. Van Essen, G.P. Wilkin, K.A. Burgomaster, A. Safdar, et al., Short-term sprint interval versus traditional endurance training: similar initial adaptations in human skeletal muscle and exercise performance, J. Physiol. 575 (3) (2006) 901–911.
[27] J. Gillen, J. Little, Z. Punthakee, M. Tarnopolsky, M. Riddell, M. Gibala, Acute high-intensity interval exercise reduces the postprandial glucose response and prevalence of hyperglycaemia in patients with type 2 diabetes, Diabetes Obes. Metab. 14 (6) (2012) 575–577.
[28] M.S. Hood, J.P. Little, M.A. Tarnopolsky, F. Myslik, M.J. Gibala, Low-volume interval training improves muscle oxidative capacity in sedentary adults, Med. Sci. Sports Exerc. 43 (10) (2011) 1849–1856, http://dx.doi.org/10.1249/mss.0b013e3182199834 (PubMed PMID: 21448086).
[29] J.P. Little, A. Safdar, G.P. Wilkin, M.A. Tarnopolsky, M.J. Gibala, A practical model of low-volume high-intensity interval training induces mitochondrial biogenesis in human skeletal muscle: potential mechanisms, J. Physiol. 588 (6) (2010) 1011–1022.
[30] K. Kim, Y.-H. Sung, J.-H. Seo, S.-W. Lee, B.-V. Lim, C.-Y. Lee, et al., Effects of treadmill exercise-intensity on short-term memory in the rats born of the lipopolysaccharide-exposed maternal rats, J. Exerc. Rehab. 11 (6) (2015) 296–302, http://dx.doi.org/10.12965/jer.150264 (PubMed PMID: PMC4697777).
[31] X.Q. Wang, G.W. Wang, Effects of treadmill exercise intensity on spatial working memory and long-term memory in rats, Life Sci. 149 (2016) 96–103, http://dx.doi.org/10.1016/j.lfs.2016.02.070 (Epub 2016/02/24. PubMed PMID: 26898129).
[32] Lou S-j, Liu J-y, H. Chang, Chen P-j, Hippocampal neurogenesis and gene expression depend on exercise intensity in juvenile rats, Brain Res. 1210 (2008) 48–55, http://dx.doi.org/10.1016/j.brainres.2008.02.080.
[33] M. Svensson, P. Rosvall, A. Boza-Serrano, E. Andersson, J. Lexell, T. Deierborg, Forced treadmill exercise can induce stress and increase neuronal damage in a mouse model of global cerebral ischemia, Neurobiol. Stress 5 (2016) 8–18, http://dx.doi.org/10.1016/j.ynstr.2016.09.002 (Epub 2016/12/17. PubMed PMID: 27981192; PubMed Central PMCID: PMCPMC5145912).
[34] K. Hayes, S. Sprague, M. Guo, W. Davis, A. Friedman, A. Kumar, et al., Forced, not voluntary, exercise effectively induces neuroprotection in stroke, Acta Neuropathol. 115 (3) (2008) 289–296, http://dx.doi.org/10.1007/s00401-008-0340-z (Epub 2008/01/23. PubMed PMID: 18210137; PubMed Central PMCID: PMCPMC2668645).
[35] D.A. Brown, M.S. Johnson, C.J. Armstrong, J.M. Lynch, N.M. Caruso, L.B. Ehlers, et al., Short-term treadmill running in the rat: what kind of stressor is it? J. Appl. Physiol. (Bethesda, Md: 1985) 103 (6) (2007) 1979–1985, http://dx.doi.org/10.1152/japplphysiol.00706.2007 (Epub 2007/10/06. PubMed PMID: 17916671).
[36] B.N. Greenwood, K.G. Spence, D.M. Crevling, P.J. Clark, W.C. Craig, M. Fleshner, Exercise-induced stress resistance is independent of exercise controllability and the medial prefrontal cortex, Eur. J. Neurosci. 37 (3) (2013) 469–478, http://dx.doi.org/10.1111/ejn.12044 (Epub 2012/11/06. PubMed PMID: 23121339; PubMed Central PMCID: PMCPMC4285393).
[37] J.R. Merritt, J.S. Rhodes, Mouse genetic differences in voluntary wheel running, adult hippocampal neurogenesis and learning on the multi-strain-adapted plus water maze, Behav. Brain Res. 280 (2015) 62–71.
[38] C.M. Yuede, S.D. Zimmerman, H. Dong, M.J. Kling, A.W. Bero, D.M. Holtzman, et al., Effects of voluntary and forced exercise on plaque deposition, hippocampal volume, and behavior in the Tg2576 mouse model of Alzheimer's disease, Neurobiol. Dis. 35 (3) (2009) 426–432, http://dx.doi.org/10.1016/j.nbd.2009.06.002 (PubMed PMID: PMC2745233).
[39] L. Prut, C. Belzung, The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review, Eur. J. Pharmacol. 463 (1) (2003) 3–33.
[40] M. Yang, J.L. Silverman, J.N. Crawley, Automated three-chambered social approach task for mice, Curr. Protoc. Neurosci. 56 (2011) 8.26.1–8.26.16 (8.26. 1-8. 16).
[41] R.M.J. Deacon, Digging and marble burying in mice: simple methods for in vivo identification of biological impacts, Nat. Protoc. 1 (1) (2006) 122–124.
[42] M. Wietrzych, H. Meziane, A. Sutter, N. Ghyselinck, P.F. Chapman, P. Chambon, et al., Working memory deficits in retinoid × receptor gamma-deficient mice, Learn. Mem. 12 (3) (2005) 318–326, http://dx.doi.org/10.1101/lm.89805 (PubMed PMID: 15897255; PubMed Central PMCID: PMC1142461).
[43] B.T. Leek, S.R. Mudaliar, R. Henry, O. Mathieu-Costello, R.S. Richardson, Effect of acute exercise on citrate synthase activity in untrained and trained human skeletal muscle, Am. J. Physiol. Regul. Integr. Comp. Physiol. 280 (2) (2001) R441–R447 (Epub 2001/02/24. PubMed PMID: 11208573).
[44] T. Milakovic, G.V. Johnson, Mitochondrial respiration and ATP production are significantly impaired in striatal cells expressing mutant huntingtin, J. Biol. Chem. 280 (35) (2005) 30773–30782.
[45] J.T. Lightfoot, M.J. Turner, M. Daves, A. Vordermark, S.R. Kleeberger, Genetic influence on daily wheel running activity level, Physiol. Genomics 19 (3) (2004) 270–276, http://dx.doi.org/10.1152/physiolgenomics.00125.2004 (Epub 2004/09/24. PubMed PMID: 15383638).
[46] V. Munive, A. Santi, I. Torres-Aleman, A concerted action of estradiol and insulin like growth factor I underlies sex differences in mood regulation by exercise, Sci. Rep. 6 (2016) 25969.
[47] B.N. Greenwood, T.E. Foley, D. Burhans, S.F. Maier, M. Fleshner, The consequences of uncontrollable stress are sensitive to duration of prior wheel running, Brain Res. 1033 (2) (2005) 164–178, http://dx.doi.org/10.1016/j.brainres.2004.11.037.
[48] B.N. Greenwood, T.E. Foley, T.V. Le, P.V. Strong, A.B. Loughridge, H.E. Day, et al.,

Long-term voluntary wheel running is rewarding and produces plasticity in the mesolimbic reward pathway, Behav. Brain Res. 217 (2) (2011) 354–362.
[49] Centers for Disease Control and Prevention, https://www.cdc.gov/physicalactivity/basics/measuring/index.html, (2015).
[50] N. Uth, H. Sørensen, K. Overgaard, P.K. Pedersen, Estimation of V̇O2max from the ratio between HRmax and HRrest – the Heart Rate Ratio Method, Eur. J. Appl. Physiol. 91 (1) (2004) 111–115, http://dx.doi.org/10.1007/s00421-003-0988-y.
[51] W.C. Miller, J. Wallace, K. Eggert, Predicting max HR and the HR-VO2 relationship for exercise prescription in obesity, Med. Sci. Sports Exerc. 25 (9) (1993) 1077–1081.
[52] T. Driss, H. Vandewalle, The measurement of maximal (anaerobic) power output on a cycle ergometer: a critical review, Biomed. Res. Int. 2013 (2013).
[53] G.A. Borg, Psychophysical bases of perceived exertion, Med. Sci. Sports Exerc. 14 (5) (1982) 377–381.
[54] W. Tsumiyama, S. Oki, N. Umei, M.E. Shimizu, T. Ono, A. Otsuka, Evaluation of the lactate threshold during downhill running in rats, J. Phys. Ther. Sci. 26 (1) (2014) 125–126.
[55] M.J. Gibala, S.L. McGee, Metabolic adaptations to short-term high-intensity interval training: a little pain for a lot of gain? Exerc. Sport Sci. Rev. 36 (2) (2008) 58–63.
[56] I. Tabata, K. Nishimura, M. Kouzaki, Y. Hirai, F. Ogita, M. Miyachi, et al., Effects of moderate-intensity endurance and high-intensity intermittent training on anaerobic capacity and VO~ 2~ m~ a~ x, Med. Sci. Sports Exerc. 28 (1996) 1327–1330.
[57] M.O. Dietrich, Z.B. Andrews, T.L. Horvath, Exercise-induced synaptogenesis in the hippocampus is dependent on UCP2-regulated mitochondrial adaptation, J. Neurosci. 28 (42) (2008) 10766–10771, http://dx.doi.org/10.1523/JNEUROSCI.2744-08.2008 (PubMed PMID: PMC3865437).
[58] J.S. Snyder, L.R. Glover, K.M. Sanzone, J.F. Kamhi, H.A. Cameron, The effects of exercise and stress on the survival and maturation of adult-generated granule cells, Hippocampus 19 (10) (2009) 898–906, http://dx.doi.org/10.1002/hipo.20552 (Epub 2009/01/22. PubMed PMID: 19156854; PubMed Central PMCID: PMCPMC2755652).
[59] S.J. Colcombe, K.I. Erickson, N. Raz, A.G. Webb, N.J. Cohen, E. McAuley, et al., Aerobic fitness reduces brain tissue loss in aging humans, J. Gerontol. A Biol. Sci. Med. Sci. 58 (2) (2003) 176–180 (Epub 2003/02/15. PubMed PMID: 12586857).
[60] S.J. Colcombe, K.I. Erickson, P.E. Scalf, J.S. Kim, R. Prakash, E. McAuley, et al., Aerobic exercise training increases brain volume in aging humans, J. Gerontol. A Biol. Sci. Med. Sci. 61 (11) (2006) 1166–1170 (Epub 2006/12/15. PubMed PMID: 17167157).
[61] J.R. Poortmans, Exercise and renal function, Sports Med. (Auckland, NZ) 1 (2) (1984) 125–153 (Epub 1984/03/01. PubMed PMID: 6567229).
[62] Ü. Pechter, M. Ots, S. Mesikepp, K. Zilmer, T. Kullissaar, T. Vihalemm, et al., Beneficial effects of water-based exercise in patients with chronic kidney disease, Int. J. Rehabil. Res. 26 (2) (2003) 153–156 (PubMed PMID: 00004356-200306000-00013).
[63] T. Fernandes, N.Y. Hashimoto, F.C. Magalhães, F.B. Fernandes, D.E. Casarini, A.K. Carmona, et al., Aerobic exercise training–induced left ventricular hypertrophy involves regulatory MicroRNAs, decreased angiotensin-converting enzyme-angiotensin II, and synergistic regulation of angiotensin-converting enzyme 2-angiotensin (1-7), Hypertension 58 (2) (2011) 182–189.
[64] Wisloff U. Wang, O.J. Kemi, Animal models in the study of exercise-induced cardiac hypertrophy, Physiol. Res. 59 (5) (2010) 633.
[65] C. Bloor, S. Pasyk, A. Leon, Interaction of age and exercise on organ and cellular development, Am. J. Pathol. 58 (2) (1970) 185.
[66] M. Shinoda, M.G. Latour, J.M. Lavoie, Effects of physical training on body composition and organ weights in ovariectomized and hyperestrogenic rats, Int. J. Obes. Relat. Metabol. Disord. 26 (3) (2002) 335–343, http://dx.doi.org/10.1038/sj.ijo.0801900 (Epub 2002/03/16. PubMed PMID: 11896488).
[67] F. Booth, J. Holloszy, Cytochrome c turnover in rat skeletal muscles, J. Biol. Chem. 252 (2) (1977) 416–419.
[68] M. Chi, C. Hintz, E. Coyle, W.H. Martin, J. Ivy, P.M. Nemeth, et al., Effects of detraining on enzymes of energy metabolism in individual human muscle fibers, Am. J. Physiol. Cell Physiol. 244 (3) (1983) C276–C287.
[69] J. Henriksson, J.S. Reitman, Time course of changes in human skeletal muscle succinate dehydrogenase and cytochrome oxidase activities and maximal oxygen uptake with physical activity and inactivity, Acta Physiol. 99 (1) (1977) 91–97.
[70] K. Klausen, L.B. Andersen, I. Pelle, Adaptive changes in work capacity, skeletal muscle capillarization and enzyme levels during training and detraining, Acta Physiol. Scand. 113 (1) (1981) 9–16, http://dx.doi.org/10.1111/j.1748-1716.1981.tb06854.x.
[71] P.M. Siu, D.A. Donley, R.W. Bryner, S.E. Alway, Citrate synthase expression and enzyme activity after endurance training in cardiac and skeletal muscles, J. Appl. Physiol. 94 (2) (2003) 555–560, http://dx.doi.org/10.1152/japplphysiol.00821.2002.
[72] E.E. Hill, E. Zack, C. Battaglini, M. Viru, A. Viru, A.C. Hackney, Exercise and circulating cortisol levels: the intensity threshold effect, J. Endocrinol. Investig. 31 (7) (2008) 587–591, http://dx.doi.org/10.1007/bf03345606 (Epub 2008/09/13. PubMed PMID: 18787373).
[73] C.H. Duman, L. Schlesinger, D.S. Russell, R.S. Duman, Voluntary exercise produces antidepressant and anxiolytic behavioral effects in mice, Brain Res. 1199 (2008) 148–158, http://dx.doi.org/10.1016/j.brainres.2007.12.047 (PubMed PMID: PMC2330082).
[74] L.J. Fulk, H.S. Stock, A. Lynn, J. Marshall, M.A. Wilson, G.A. Hand, Chronic physical exercise reduces anxiety-like behavior in rats, Int. J. Sports Med. 25 (01) (2004) 78–82, http://dx.doi.org/10.1055/s-2003-45235.
[75] E. Binder, S.K. Droste, F. Ohl, J.M.H.M. Reul, Regular voluntary exercise reduces anxiety-related behaviour and impulsiveness in mice, Behav. Brain Res. 155 (2) (2004) 197–206, http://dx.doi.org/10.1016/j.bbr.2004.04.017.
[76] J.N. Salam, J.H. Fox, E.M. DeTroy, M.H. Guignon, D.F. Wohl, W.A. Falls, Voluntary exercise in C57 mice is anxiolytic across several measures of anxiety, Behav. Brain Res. 197 (1) (2009) 31–40, http://dx.doi.org/10.1016/j.bbr.2008.07.036.
[77] P.J. Carek, S.E. Laibstain, S.M. Carek, Exercise for the treatment of depression and anxiety, Int. J. Psychiatr. Med. 41 (1) (2011) 15–28.
[78] G.L. Stonerock, B.M. Hoffman, P.J. Smith, J.A. Blumenthal, Exercise as treatment for anxiety: systematic review and analysis, Ann. Behav. Med. 49 (4) (2015) 542–556.
[79] A.M. Basso, M. Spina, J. Rivier, W. Vale, G.F. Koob, Corticotropin-releasing factor antagonist attenuates the “anxiogenic-like” effect in the defensive burying paradigm but not in the elevated plus-maze following chronic cocaine in rats, Psychopharmacology 145 (1) (1999) 21–30, http://dx.doi.org/10.1007/s002130051028.
[80] S.F. De Boer, J.M. Koolhaas, Defensive burying in rodents: ethology, neurobiology and psychopharmacology, Eur. J. Pharmacol. 463 (1) (2003) 145–161, http://dx.doi.org/10.1016/S0014-2999(03)01278-0.
[81] A. Thomas, A. Burant, N. Bui, D. Graham, L.A. Yuva-Paylor, R. Paylor, Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety, Psychopharmacology 204 (2) (2009) 361–373, http://dx.doi.org/10.1007/s00213-009-1466-y.
[82] L.M. Gorton, M.G. Vuckovic, N. Vertelkina, G.M. Petzinger, M.W. Jakowec, R.I. Wood, Exercise effects on motor and affective behavior and catecholamine neurochemistry in the MPTP-lesioned mouse, Behav. Brain Res. 213 (2) (2010) 253–262, http://dx.doi.org/10.1016/j.bbr.2010.05.009 (PubMed PMID: PMC2902645).
[83] J. Young, M. Angevaren, J. Rusted, N. Tabet, Aerobic exercise to improve cognitive function in older people without known cognitive impairment, Cochrane Libr (4) (2015) (CD005381).
[84] C.K. Barha, R.S. Falck, J.C. Davis, L.S. Nagamatsu, T. Liu-Ambrose, Sex differences in aerobic exercise efficacy to improve cognition: a systematic review and meta-analysis of studies in older rodents, Front. Neuroendocrinol. 46 (2017) 86–105.
[85] K. Inoue, Y. Hanaoka, T. Nishijima, M. Okamoto, H. Chang, T. Saito, et al., Long-term mild exercise training enhances hippocampus-dependent memory in rats, Int. J. Sports Med. 36 (04) (2015) 280–285.
[86] R.C. Cassilhas, S. Tufik, M.T. de Mello, Physical exercise, neuroplasticity, spatial learning and memory, Cell. Mol. Life Sci. 73 (5) (2016) 975–983.
[87] G. Kennedy, R.J. Hardman, H. Macpherson, A.B. Scholey, A. Pipingas, How does exercise reduce the rate of age-associated cognitive decline? A review of potential mechanisms, J. Alzheimers Dis. 55 (1) (2017) 1–18.
[88] L. Bherer, K.I. Erickson, T. Liu-Ambrose, A review of the effects of physical activity and exercise on cognitive and brain functions in older adults, J. Aging Res. 2013 (2013).
[89] M.E. Bach, R.D. Hawkins, M. Osman, E.R. Kandel, M. Mayford, Impairment of spatial but not contextual memory in CaMKII mutant mice with a selective loss of hippocampal LTP in the range of the θ frequency, Cell 81 (6) (1995) 905–915.
[90] J.P. Aggleton, P.R. Hunt, J.N.P. Rawlins, The effects of hippocampal lesions upon spatial and non-spatial tests of working memory, Behav. Brain Res. 19 (2) (1986) 133–146, http://dx.doi.org/10.1016/0166-4328(86)90011-2.
[91] R. Lalonde, The neurobiological basis of spontaneous alternation, Neurosci. Biobehav. Rev. 26 (1) (2002) 91–104, http://dx.doi.org/10.1016/S0149-7634(01)00041-0.