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Selection for aerobic capacity affects corticosterone, monoamines and wheel-running activity

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Abstract

A positive genetic relationship between aerobic capacity and voluntary exercise has been suggested from earlier studies of mice selected for increased wheel-running activity. To further investigate the relationship between aerobic capacity and exercise behavior, wheel-running activity was studied in female rats bidirectionally selected for intrinsic aerobic capacity (high capacity runners — HCR; low capacity runners — LCR). Aerobic capacity was measured using a forced treadmill paradigm; the subpopulations of animals used in this experiment exhibited a 471% difference in endurance capacity. Rats were housed individually, with or without access to running wheels. Wheel-running activity was recorded and analyzed from weeks two through seven during an eight-week trial to determine voluntary activity levels. HCR animals exhibited 33% greater total wheel-running distance per day compared to LCR rats (16,838.7+1337.30 m versus 12,665.8+893.88 m), which was due to the HCR rats exhibiting increases in both running speed and duration over LCR rats. Differences in the intermittency of wheel running were also observed. HCR rats engaged in more bouts of running per day than LCR rats, and trended towards running faster, for more time, and for longer distances during bouts of running than LCR rats. Following the running trial, measurement of plasma corticosterone concentration and striatal dopaminergic activity showed differences between HCR and LCR rats, suggesting a divergence of physiological systems that could potentially influence locomotor behaviors in these lines. These results are consistent with earlier work, and suggest an evolutionarily conserved relationship between physiological capacity and behavioral activity of exercise.

Keywords: Artificial selection; Correlated response; Endurance capacity; Voluntary exercise; Corticosterone; Dopamine

1. Introduction

The physiological capacity and motivational behavior to exercise are considered multifactorial traits, implying influence by genetic and environmental factors. As a result of the polygenic and pleiotropic features of these complex traits, it is likely that the associated genes could simultaneously affect two seemingly disparate traits. A genetic correlation between key physiological characteristics and the expression of behavior suggests shared or common mechanisms [1]. Human twin studies demonstrate that a substantial genetic component exists for both the ability to perform aerobic exercise [2] as well as a propensity to engage in exercise [3]. Whether a genetic correlation between one's level of aerobic capacity and tendency to exercise exists is unclear.

Animal models generated from artificial selection are tools that can be used to gain a better understanding of the genetic suite that forms the variation for complex traits [4,5]. The usual goal of selective breeding is to change the mean value of a trait in a defined population, compared to a control population. In theory, divergent artificial selection for a complex trait produces somewhat ideal genetic models because contrasting allelic variation segregates at the extremes from one generation to the next. In addition, the selection process often carries the phenotypic means for each line beyond the range of the extremes

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within the founder population [6]. By maintaining a high level of heterozygosity at each generation during selection, the main complement of allelic variants causative of trait difference is concentrated within each line. If gene overlap or pleiotropy is present, artificial selection for one trait can result in a correlated response to selection for other traits [4,7].

Over the past decade, large-scale artificial selective breeding has been used to develop two different models of physical activity in rodents [8,9]. In the first model, Swallow et al. [9] used voluntary wheel-running activity as a selection criterion to establish replicate lines of outbred mice (Hsd:ICR strain) that differed in voluntary exercise activity levels. After 10 generations of selection, high activity lines exhibited a 70% increase in wheel-running behavior (total number of ~1 meter revolutions run per day) relative to controls; [9]. High activity lines also differed in a variety of physiological traits compared to controls [10–13], including a 7% increase in maximal aerobic capacity (VO_2max) [12,14] , intermediate differences in skeletal muscle metabolism (indicated by elevated glucose uptake levels; [15], and increased mitochondrial and glycolytic enzyme levels [16]. Taken together, these results support an association between aerobic capacity and increased voluntary activity.

The second model, generated by Koch and Britton [8], resulted in lines of rats that diverged in untrained aerobic running capacity by using bidirectional artificial selection for forced treadmill running capacity on a widely heterogeneous N-NIH stock base population. After 10 generations, rats bred as low capacity runners (LCR) and high capacity runners (HCR) differed by 317% in treadmill running capacity [8]. The selected lines also diverged for VO_2max, economy of running, left ventricular cell contractility, and skeletal muscle oxidative enzyme activity [17].

Physiological responses to physical activity include the release of glucocorticoids such as corticosterone [18], which helps to stimulate the release of energy stores in the body, such as glycogen and fat, allowing the animal to exercise [19]. These hormones also initiate a psychological response to environmental stressors via receptors in a variety of brain regions such as the amygdala and hippocampus [20]. Blood plasma levels of corticosterone can provide information regarding an animal's level of stress, and also the extent of physical exertion [21]. Both acute and long term voluntary wheel running affect plasma concentrations of corticosterone [22,23], however previous data are not always in agreement (see [22]). Selective breeding for endurance capacity could have dramatic effects on resting or exercise induced plasma corticosterone levels due to the role of this hormone in the mobilization of energy, and maintenance of physical activity [19], and these changes could influence other physiological or psychological systems.

Previous studies have shown that central dopaminergic activity affects levels of wheel-running behavior [24]. For example, depletion of dopamine (DA) in the nucleus accumbens can lead to a decrease in the performance of energetically expensive activity [24], and the age related decreases in physical activity seen in many rodent species are associated with decreases in dopamine release and receptor expression in the forebrain [25]. Reciprocally, voluntary exercise can affect central dopaminergic activity. Treadmill running increases DA release and dopamine type-2 (D_2) receptor levels in the nucleus accumbens [24]. Dopaminergic activity in the striatum is also affected by physical activity, with reduced levels of striatal DA and D_2 receptor expression observed with decreases in physical activity, and increased levels of striatal DA following forced treadmill running [24]. Exercise mediated changes in neurotransmitter function also affect a variety of traits such as anxiety, depression, and motivational drive (reviewed by [26,27]). The effect of the striatal dopaminergic system on motivational drive is thought to play a role in determining an individual's endurance capacity [28]. Supporting this idea, mice selected for increased voluntary wheel-running behavior [9] not only experience an increase in aerobic capacity [14], but also exhibit an increased expression of the immediate early gene *Fos* in the striatum, indicating increased activity in this brain area [13].

The purpose of this study was to test the hypothesis that selection for aerobic capacity results in a correlated response in voluntary exercise activity, changes in corticosterone response to exercise, and altered dopaminergic activity in the striatum. Rats derived from the LCR and HCR colony were housed with or without running wheels for an eight-week period, and activity levels were recorded from weeks two through seven. Following this period, tissue was collected to analyze levels of plasma corticosterone and central dopaminergic activity. The data show a difference in voluntary activity levels between groups of rats bred for low and high intrinsic aerobic capacity. However, within selected lines, there is no association between aerobic capacity and level of voluntary activity. Microanalysis of wheel-running activity revealed behavioral differences in running periodicity between LCR and HCR rats. These two populations exhibit different corticosterone responses to 8 weeks of wheel running, with HCR animals exhibiting decreased levels of plasma corticosterone compared to LCR only after this running period. Additionally, HCR animals exhibited higher levels of striatal dopaminergic activity than LCR animals, but diverged in their response to running wheel access.

2. Materials and methods

2.1. Animals

A previous report gives a detailed description on the development of the rat models for aerobic exercise capacity [8]. In summary, bidirectionally selected lines were generated from a founder population of 80 male and 88 female N-NIH stock rats based on intrinsic aerobic treadmill running capacity. Thirteen families for each line were set up for a within-family rotational breeding paradigm. This schedule permits <1% inbreeding per generation to maintain a heterogeneous substrate within each selected line.

At each generation young adult rats (11 weeks of age) were tested for their inherent ability to perform forced speed-ramped treadmill running until exhausted. This test was performed daily over five consecutive days. The greatest distance in meters (m) achieved out of the five trails was considered the best estimate of an individual's aerobic exercise capacity [8]. The highest scored female and male from each of the thirteen families were

selected as breeders for the next generation of high capacity runners (HCR). The same process was used with lowest scored females and males to generate low capacity runners (LCR).

It is important to note that no control group was maintained with these selected lines of animals. A full explanation for this method is given by Koch and Britton [8]. It should be stated that all analyses performed using these animals compare the lines to each other, and do not represent a divergence from wild-type rats.

The rats used in the current study were females derived from generations nine and ten of selection. Distance run to exhaustion for LCR animals was 208.34±8.28 (mean±S.E.), and 1005.65±35.59 m (mean±S.E.) for HCR rats. Animals were air shipped to the University of South Dakota (Vermillion, SD) animal facility at approximately 21 weeks of age.

2.2. Voluntary running study

All procedures were carried out according to the *NIH Guide for the Care and Use of Laboratory Animals.* The following protocol was approved by the Institutional Animal Care and Use Committee of the University of South Dakota. Upon arrival at our facility, rats were housed individually in clear plastic Nalgene™ cages (43"×27"×15") with cedar bedding and wire lids. Teklad Rodent Diet (8604) and water were available *ad libitum*. Rats were maintained on a 12:12 reversed dark:light cycle (dark 0900–2100) using an automatic timer. Body mass was measured upon arrival, and on day 6 of every week at the end of the dark period (between 2000 and 2100). During measurement of body mass, each animal's bedding, food and water were changed. Rats were given 20 days to acclimate to this environment before introduction of the running wheel.

Following the acclimation period, rats from both strains were randomly assigned to a wheel group ($n=17$ LCR, 12 HCR) or a sedentary group ($n=16$ LCR, 15 HCR). Animals in the wheel group had free access to an approximately 1.08 m circumference wheel (Nalgene™ Activity Wheels for Rats) which was introduced into the cage during body mass assessment. Animals in the 'sedentary' group did not receive a wheel and were used as controls to determine the effects of the presence of a running wheel on body mass, endocrine and neurochemical function.

Running wheels were connected to a Minimitter™ system that used a magnetic sensor to record wheel rotations. This system was interfaced with a computer that used VitalView™ software to record wheel running at 1 min intervals 24 h/day for 56 days. Due to data collection errors, running data from days one through seven and 50 through 56 of this trial were omitted, thus only data from days eight through 49 appear in the data analysis.

The circumference of the running wheels used was 1.084 m. VitalView™ software (Mini Mitter Co., Inc., Bend, OR) was set to store the number of revolutions in 1 min bins. Using this information, the number of meters run each minute as well as running speed, in meters per minute, was calculated. The number of bins with non-zero values were recorded as 1 min running values and used to ascertain the total amount of time each rat spent running [9].

A microanalysis of running behavior periodicity was performed using ClockLab™ software (Actimetrics, Wilmette, IL) to build and interpret an actogram of running behavior. This software determines wheel running intermittency by recognizing bouts of running activity. ClockLab™ recognizes a running bout as at least one non-zero bin (bin=1 min) of wheel running, separated from other non-zero bins by at least 1 min of no running (zero-value bin). These data were used to calculate the number of bouts run per day, the length of each running bout in time and in distance, and the average speed of each running bout.

Between days 42 and 49 of wheel access, rats were monitored for stages of the estrous cycle by assessing the cytology of daily vaginal lavages (performed between 2000–2100 h) with 0.9% saline via an eyedropper. This was done to ensure that all rats were killed during diestrous II, to control for hormonal variability for measurements of neurochemistry. Although the lavage process only required approximately 20 s of handling per rat, it is possible that the procedure may have had some impact on running behavior (see Results).

Rats were killed between 1000 and 1200 h, on days 57, 58, 59 and 60 of the experiment. This allowed all animals at least 56 days of voluntary wheel running; all animals were killed in diestrous II. Wheels were not removed from the cages on day 57 as this could result in significant stress to animals housed with wheels. Half of each group of animals was subjected to 1 h of restraint stress immediately before being killed. Data from these animals are not included in the plasma corticosterone or monoamine analyses presented herein; therefore the numbers in these analyses are as follows (7 LCR, SEDENTARY; 7 LCR, WHEEL; 3 HCR, SEDENTARY; 6 HCR, WHEEL). Rats were killed via rapid decapitation with a guillotine. Trunk blood was collected using a clean glass funnel and clean glass tests tubes, and then centrifuged to separate blood plasma. Blood plasma was stored in 1.5 mL microcentrifuge tubes at −80 °C until analyzed. Whole brains were removed immediately upon decapitation and frozen on dry ice. Brains were then stored at −80 °C until neurochemical analysis.

2.3. Corticosterone sampling

Plasma corticosterone concentration was measured using a corticosterone enzyme-linked immunoassay (ELA) kit, following instructions from the manufacturer (R&D Systems, Minneapolis, MN, USA). We diluted 10 μL of plasma and 0.5 μL steroid displacement reagent in 990 μL of assay buffer, completing a 100-fold dilution. Plasma samples from each animal were added in duplicate to plate wells, coated with donkey anti-sheep polyclonal B antibody. Standard concentrations of B were added in duplicate to 14 wells in the following concentrations: 20,000 pg/mL, 10,000 pg/mL, 6666.7 pg/mL, 4000 pg/mL, 800 pg/mL, 160 pg/mL, and 32 pg/mL. Fifty microliters of B (200,000 pg/mL) conjugated to alkaline phosphatase and 50 μL of antibody solution (sheep polyclonal antibody to B) were added to each well containing animal samples. Plates were then incubated at room temperature for 2 h on a horizontal shaker.

After incubating for 2 h, an automated plate washer (Bio-Tek Instruments, Winooski, VT, USA) was used to wash the assay plate. We then added 200 μL of *p*-nitrophenyl phosphate (PNP) substrate to each well in the plate. Following the addition of

PNP, plates were incubated for 1 h at room temperature. After this incubation, 50 μL of trisodium phosphate solution was added to end the reaction. The plate was then placed into an automated microplate reader (Bio-Tek Instruments).

Detection of plasma corticosterone concentration was performed by measuring the absorbance of samples at 405 nm (wavelength correction set at 595 nm) with automated plate reader software (KinetiCalc Jr., Bio-Tek Instruments). From the absorbance values obtained from samples, we calculated maximum binding percentages, which averaged 14.8%, and non-specific binding percentage, which was 5.2%; both of which were within the manufacturer's range. The detection limit sensitivity of this assay was 27.0 pg/mL.

2.4. Monoamine concentrations and activity

The frozen brains were serially sectioned at 300 μm intervals in an IEC cryostat (−10 °C) and thaw mounted on glass slides. The striatum was microdissected using a freezing plate and a dissecting microscope as described by Palkovitz and Brownstein [29]. All brains were done in the same session to eliminate any effect of procedure on monoamine concentration. Dopamine (DA) and dihydroxyphenyacetic acid (DOPAC) were measured using high performance liquid chromatography with electrochemical detection as described by Renner and Luine [30,31]. Tissue samples were expelled into 60 μL of sodium acetate buffer (pH 5) containing 0.5×10^{-7} M dihydroxybenzylamine (DHBA; internal standard) and freeze–thawed. After centrifugation (15,000 ×g for 2 min), 2 μL of a 1 mg/10 mL H_2O ascorbate oxidase solution (Boehringer Mannheim, UK) was added to each sample [32]. The supernatant was removed and 45 μL was injected into a Waters chromatography system (Waters Associates, Milford, MA, USA) and analyzed electrochemically with an LC-4B potentiostat and a glassy carbon electrode (Bioanalytical Systems, Inc., West Lafayette, IN, USA). The electrode potential was set at +0.65 V with respect to an Ag/AgCl reference electrode. Separation was accomplished using a 4 μm C-18 radial compression cartridge (Waters Associates). The mobile phase consisted of 11 g citric acid, 8.6 g sodium acetate, 110 mg octylsulfonic acid (Sigma Chemical Co., St Louis, MO, USA), 250 mg EDTA and 100 mL methanol in 1 L of water. The tissue pellets were dissolved in 0.3 N NaOH and analyzed for protein content [33].

The concentrations of the amines and amine metabolites were calculated with respect to peak height values obtained from standard runs set in the internal standard mode using the CSW32 data program (DataApex Ltd., Czech Republic). Corrections were programmed for injection volume versus preparation volume. The resulting pg values are divided by μg protein to yield pg amine/μg protein. Ratios of monoamine/metabolite are used to estimate neuronal activity. These are presented as a unit-less ratio.

2.5. Statistical analysis

Two-way repeated measures ANOVA (RMANOVA; between subjects factor — Selection; within subjects factor — Day) was used to analyze running data. Measures of running activity include meters run per day, minutes run per day, and average running speed (m/min). Comparisons of the length of bouts (m), bout speed (m/min), duration of bouts (min) and the number of bouts per day for HCR and LCR were also performed using RMANOVA (between subjects factor — Selection; within subjects factor — Day). A Pearson Correlation was performed to compare the average weekly running activity and endurance capacity scores of all animals. Initial and final body mass as well as total weight gain of LCR and HCR animals were compared using one-way ANOVA (factor=Line); three way RMANOVA was used to analyze the effects of selection for endurance capacity and running wheel access on body mass throughout the experiment (between subject factor — Selection, Wheel Access; within subject factor — Week). Analysis of plasma corticosterone concentrations, striatal DA, striatal DOPAC and the ratio of DA to DOPAC (DOPAC/DA) was accomplished with two-way ANOVA (Selection × Wheel Access), followed by Student's *t*-test when a significant interaction was observed. All values are presented as mean±S.E. Confidence levels of $P\leq0.05$ were assigned for statistical significance.

In our analysis, we observed two behavioral outliers, with regard to wheel running, in the HCR group. Fig. 1 is presented showing individual running behavior of all animals in the HCR group. For all running analyses, these two animals are omitted.

3. Results

3.1. Intrinsic endurance measures

Animals were phenotyped for endurance capacity, determined by distance run to exhaustion, at 11 weeks of age. These data (mean±S.E.) for all groups are presented in Table 1.

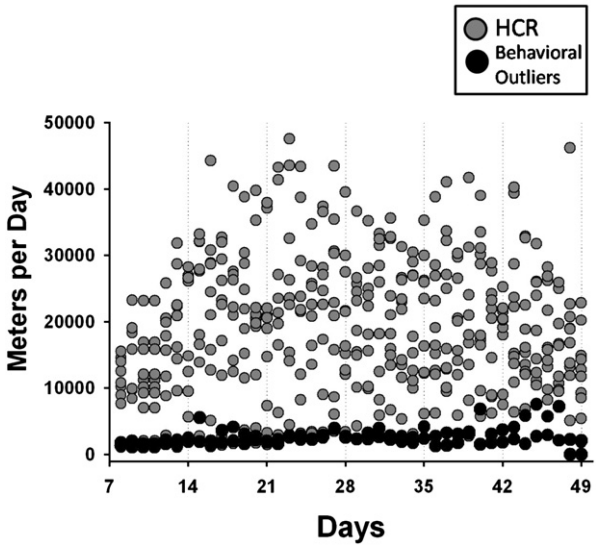

Fig. 1. Individual running data (meters run per day) for HCR animals. Note two individuals who express consistently aberrantly low levels of wheel running.

Table 1
Intrinsic endurance capacity, estimated by distance run to exhaustion (m). HCR animals ran significantly longer to exhaustion than LCR animals ($P<0.001$)

	HCR		LCR	
N	15	12	16	17
Treatment	Sedentary	Wheel	Sedentary	Wheel
Distance to exhaustion	1017.35±43.91	991.84±53.85	206.34±14.12	210.22±9.49

3.2. Global analysis of wheel running

Weekly averages for global wheel-running data (distance, speed and time) of LCR and HCR rats are summarized in Table 2. During the second week of wheel access (days 8 through 14) HCR rats ran 12,758.4±2616.02 m/day compared with LCR rats running 7312.0±1620.15 m/day. The difference in running distance between the lines decreased during the course of the experiment as a result of the LCR rats increasing their running activity at a higher rate than HCR rats. The average daily running distance over the entire 42 day recorded running period was 16,838.7±1337.30 m for HCR rats compared to 12,665.8±893.88 m for LCR animals, a difference of 33%. From days 42 through 49, running intensity and duration was highly variable, and both lines of rats showed an overall decrease in wheel-running activity.

Using RMANOVA, we show that HCR rats spend more time engaged in wheel running ($F(1,25)=6.513$;$P=0.017$; Fig. 2a), and show a slight trend towards running faster than LCR rats ($F(1,25)=2.584$;$P=0.120$; Fig. 2b) over the 42 days analyzed. Combined, these differences affected total distance run per day; HCR animals ran significantly more than LCR animals ($F(1,25)=4.495$;$P=0.044$; Fig. 2c) during the experiment.

3.3. Individual correlations

Individual levels of voluntary wheel running were highly repeatable over the 42 days analyzed. Pearson correlation of weeks two through seven revealed that both HCR and LCR individuals maintain their relative levels of total running during the experiment (Table 3). A Pearson correlation was also performed to relate endurance scores and voluntary running activity levels of each individual. Pearson correlations on endurance capacity scores and total average voluntary wheel running indicate that individual endurance scores are not related to wheel-running distance ($r=0.164$; $P=0.396$), duration ($r=0.232$; $P=0.226$), or running intensity ($r=0.069$; $P=0.721$) within individuals.

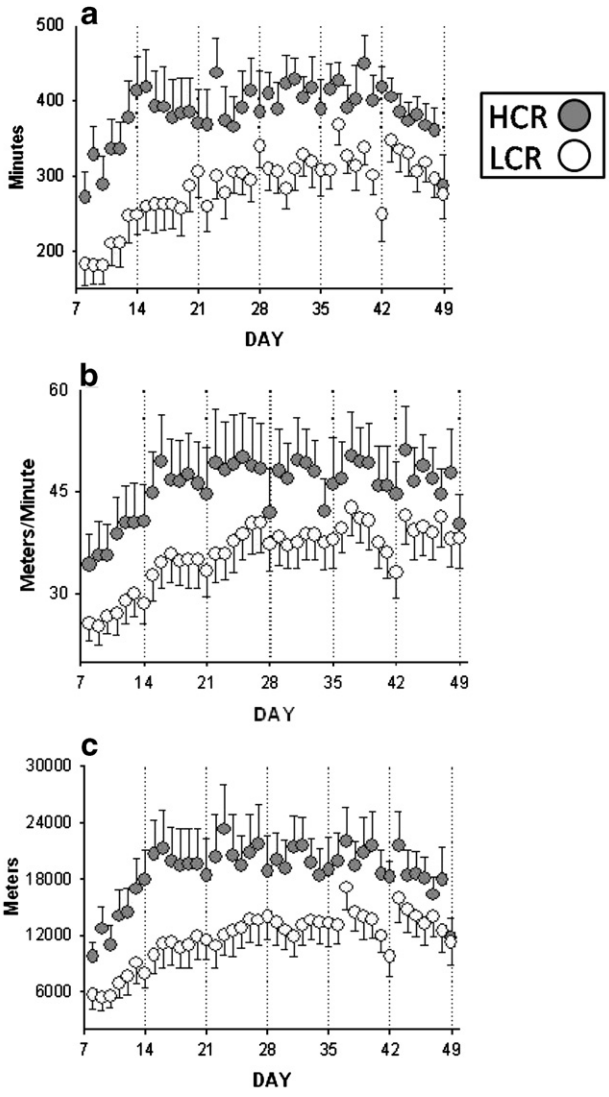

Fig. 2. (a–c). Measures of wheel-running behavior of HCR and LCR animals (mean±S.E.). HCR animals ran more minutes per day ($P<0.017$; a) trend towards higher speeds ($P=0.120$; b) than LCR animals. This resulted in HCR animals running more total distance throughout the experiment ($P<0.044$; c).

Table 2
Weekly averages for global wheel-running data (mean±S.E.)

Week	Distance (m/day)		Speed (m/min)		Time (min/day)	
	HCR	LCR	HCR	LCR	HCR	LCR
2	12758.4±2615.98	7311.9±1620.11	36.7±5.59	30.0±3.14	303.5±37.2	206.8±26.2
3	18338.0±3859.16	11057.9±2362.39	44.9±6.64	37.6±4.05	349.0±46.4	269.7±31.2
4	19136.0±4212.11	12797.3±2491.88	44.1±6.85	41.5±4.27	353.0±44.3	296.1±27.2
5	18389.9±3261.38	13087.5±2165.62	45.5±6.00	41.9±3.82	368.2±37.2	307.7±24.7
6	18573.6±3219.26	13423.7±2001.72	45.6±6.17	42.5±3.88	374.6±35.4	313.0±22.2
7	14858.2±2558.30	12787.9±2103.19	44.2±5.50	39.4±3.98	297.8±24.2	292.9±24.0

HCR rats exhibited higher levels of meters run per day ($P<0.017$), running speed ($P=0.120$), and minutes run per day ($P=0.044$) than LCR rats.
Values are mean±S.E. $n=12$—HCR; 17—LCR.

Table 3
Pearson correlation of endurance capacity and weekly running distance

HCR wheel-running distance							LCR wheel-running distance						
	Week							Week					
	2	3	4	5	6	7		2	3	4	5	6	7
Endurance Capacity	0.257	0.252	0.268	0.425	0.425	0.297	Endurance capacity	0.039	0.029	0.115	0.338	0.211	0.419
Week 2		0.940*	0.896*	0.948*	0.942*	0.879*	Week 2		0.924*	0.933*	0.761*	0.737*	0.618*
Week 3			0.986*	0.948*	0.920*	0.916*	Week 3			0.956*	0.848*	0.786*	0.628*
Week 4				0.923*	0.905*	0.938*	Week 4				0.876*	0.845*	0.672*
Week 5					0.980*	0.937*	Week 5					0.980*	0.805*
Week 6						0.933*	Week 6						0.884*

Individual measures of endurance capacity and voluntary wheel running throughout the experiment.
2-tailed significance. (* = $P<0.01$). $n=12$—HCR; $n=17$—LCR.

3.4. Microanalysis of wheel running

Analysis of running bout activity revealed that HCR animals engaged in more running bouts per day ($F(1,25)=5.760$; $P=0.024$; Fig. 3a), and exhibit a trend towards running for longer times ($F(1,25)=2.847$; $P=0.104$; Fig. 3b), at higher speeds ($F(1,25)=3.515$; $P=0.073$; Fig. 3c), and for longer distances ($F(1,25)=2.673$; $P=0.115$; Fig. 3d) during these bouts than LCR animals.

3.5. Body mass

As previously reported [8], LCR animals had a greater body mass compared to HCR rats (Fig. 4). Upon arrival, LCR animals were heavier than HCR animals ($F(1,59)=55.501$; $P<0.001$). Three-way RMANOVA indicated a larger average body mass of LCR throughout the experiment ($F(1,56)=66.204$; $P<0.001$), and that the average body mass of both lines increased each successive week ($F(8,56)=170.109$; $P<0.001$). There was no interaction between wheel access, selected line, and week ($F(8,56)=1.054$; $P=0.309$). Immediately before animals were killed, they were weighed; LCR showed an overall gain of 25.83±3.30 g, while HCR rats gained 29.92±3.08 g during the experiment; these values did not significantly differ ($F(1,59)=0.794$; $P=0.377$). At the end of the experiment, LCR rats (252.27±3.40) weighed more than HCR rats (219.98±3.83) ($F(1,59)=60.783$; $P<0.001$).

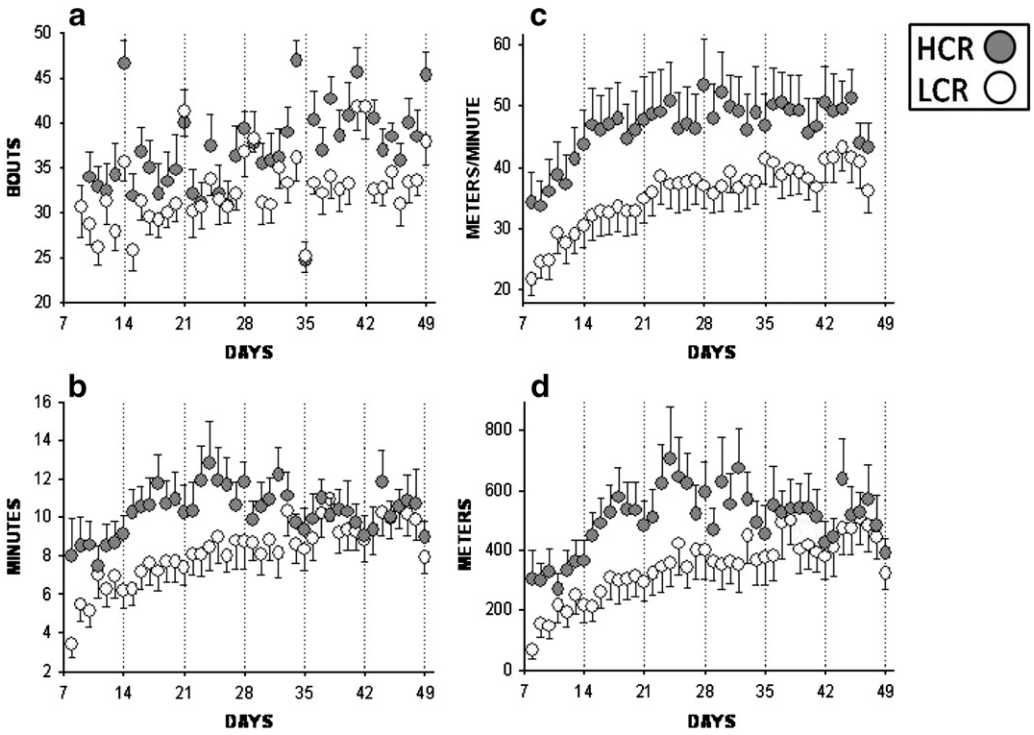

Fig. 3. (a–d). Microanalysis of running behavior revealed differences in the periodicity of running in the HCR and LCR animals (mean±S.E.). HCR animals engaged in more bouts per day ($P<0.024$; a), trend towards more minutes ($P<0.104$; b) and higher speeds ($P<0.073$; c) during bouts. These changes resulted in a trend towards more meters run per bout ($P=0.115$; d).

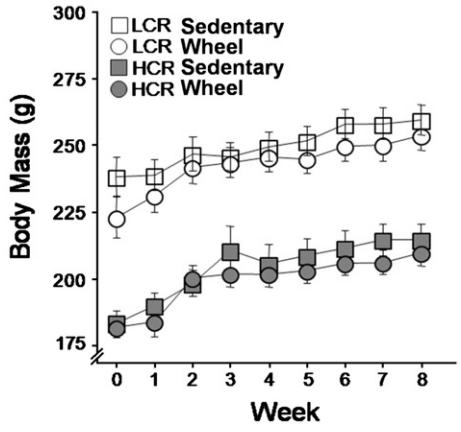

Fig. 4. Body mass was measured for all animals weekly (mean±S.E.). LCR animals weighed more than HCR animals throughout the 8 week experiment ($P<0.01$). Presence of a running wheel had no effect the body mass ($P=0.187$).

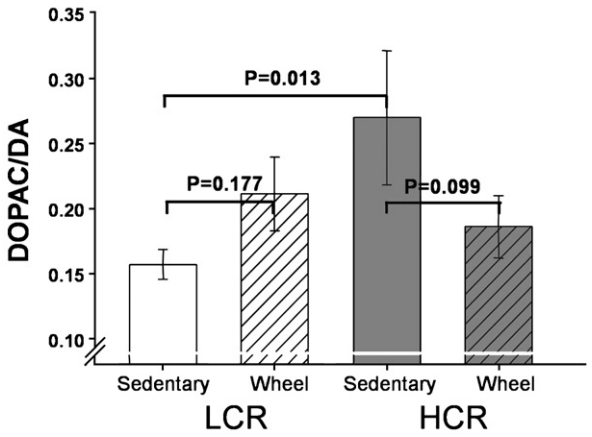

Fig. 6. (mean±S.E.). Dopaminergic activity (DA/DOPAC) was higher in HCR animals than in LCR animals housed without a wheel ($P=0.013$). Eight weeks of voluntary wheel running eliminated this difference ($P=0.50$).

3.6. Plasma corticosterone

Bidirectional selection for endurance capacity resulted in differences in plasma corticosterone response to wheel running (Selection × Wheel; $F(3,14)=5.400$; $P=0.036$; Fig. 5). Levels of plasma corticosterone concentration were similar in sedentary LCR and HCR animals, however following 8 weeks of access to a running wheel, HCR animals exhibit lower levels of plasma corticosterone than LCR animals in the same condition ($t(7)=3.176$; $P=0.016$).

3.7. Monoamines

Response of striatal dopaminergic activity (DOPAC/DA) to long term access to a running wheel diverged in HCR and LCR animals (Fig. 6). A significant interaction was observed between selection and wheel access ($F(1,22)=5.358$; $P=0.031$). Post hoc analysis indicated a trend toward decreased dopaminergic activity in HCR animals ($t(8)=1.865$; $P<0.099$), and a slight trend toward an increase in LCR animals ($t(13)=1.432$; $P<0.177$). Striatal dopaminergic activity differed between selected lines without access to running wheels; HCR animals expressed higher levels of striatal dopaminergic activity than LCR animals ($t(12)=2.712$; $P<0.013$). The presence of a running wheel abolished this difference ($t(9)=0.697$; $P=0.500$).

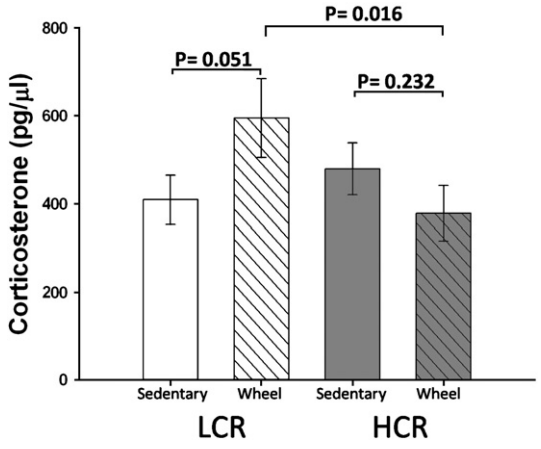

Fig. 5. Plasma corticosterone concentration for HCR and LCR animals in sedentary and wheel-running conditions. A significant interaction was observed between selection and wheel running ($P=0.036$). No difference was observed in plasma corticosterone levels of sedentary LCR and HCR animals. Following running wheel access, HCR exhibited decreased plasma corticosterone compared LCR animals ($P=0.016$).

4. Discussion

In this study, we show that voluntary wheel-running activity responds positively to bidirectional selection for intrinsic treadmill running capacity, in support of our hypothesis that a genetic correlation exists between exercise behavior and aerobic capacity level. Previously, we [14] showed that mice selected for increased wheel running exhibited a 75% increase in activity levels and a 6% correlated response in VO_2max following 10 generations of selection. In the present study, we found that 10 generations of bidirectional selection for treadmill endurance capacity resulted in a 471% difference in treadmill running capacity, and a 35% difference in total voluntary wheel-running activity. In both of these selection studies, the magnitude of the response of the unselected trait is approximately 8% of the response of the selected trait. The consistency of this relationship, both qualitative and quantitative, strongly supports a genetic link between these traits.

Several previous studies using inbred strains of rodents have been useful to help estimate the genetic variability for wheel-running activity [34–36] and treadmill running capacity [37,38]. Lerman et al. [34] show wide genetic variation for both forced treadmill and voluntary wheel running exists among seven phylogenetically different inbred mouse strains. Although they report no apparent relationship between wheel running and treadmill running within these strains, one genotype (Swiss Webster strain) demonstrated high performances for both traits. Likewise, Friedman et al. [39] tested a more heterogeneous population of mice (random bred ICR strain) and found both voluntary wheel-running and forced sprint running speed to be positively correlated with the physiological measurement of

aerobic capacity (VO_2max). The overall results of these previous studies in unselected populations reinforce the possibility that there are many combinations of allelic variants that contribute to the variation for both voluntary and forced exercise.

Models derived via selective breeding are more appropriate than the previously mentioned models to address genetic correlations between exercise behavior and physiological capacity. First, in selected lines, the alleles that influence the selected trait are concentrated, leaving heterogeneity within the rest of the genome. Second, most artificial selection paradigms maintain low levels of inbreeding in order to maintain a genetic substrate for selection. Both of these elements of selective breeding preserve high variation among traits not genetically correlated with the selected trait. Third, bidirectional selection for a quantitative trait, such as exercise capacity, is performed multiple times across several generations. Only organisms that perform "the best" for a given trait are selected as breeders at each generation. This not only ensures that the genetic component of selected organisms truly represents each end of the spectrum, but also this method selects for a lack of sensitivity to subtle differences in laboratory and institutional settings across time.

For both HCR and LCR groups, wheel-running behavior increased from week two to week four and then reached a plateau. HCR rats ran at levels higher than LCR beginning at the start of the experiment, and then continued to show higher mean levels of running distance, running intensity and running duration through day 42. During days 43 through 49 this trend changed and HCR rats ran at levels approximately equal to LCR rats. In both lines, rat running behavior became erratic during this time with a greater decline in total wheel running by HCR compared to LCR rats. It is not clear why running behavior changed during days 43 through 49, although it seems unlikely that the decrease is part of a natural progression for running behavior. During this week, we performed vaginal lavages on all rats to assess estrous cycle stages for neurochemical studies. Therefore, either the stress or the time cue from this procedure may have been disruptive to their normal behavioral rhythms [40].

A difference in wheel-running activity can be explained by a difference in either wheel-running speed and/or time spent running on the wheels. In mice, lines selected over 35 generations for high wheel-running behavior exhibit 170% greater wheel-running distance compared to controls resulting from increased running speeds, but no change in time spent running [9]. Similarly, Dohm et al. [41] attribute the higher levels of running activity in wild mice over laboratory bred mice (ICR strain) to greater running intensity with no difference in duration of activity. In our study, HCR rats exhibit not only greater levels of voluntary wheel-running intensity, but also higher running durations compared to LCR rats (Table 2; Fig. 2).

A study by Rodnick et al. [42] investigated the components for voluntary activity of wild-type Sprague–Dawley rats which exhibit a large amount of variability in untrained voluntary wheel running. Individual rats that initially expressed high levels of voluntary wheel running (12.6 km/day) ran four times the amount of a group of rats with an initial low activity level score (3.1 km/day) over a three-week period. This difference was due to differences in the duration of running (278 min/day versus 75 min/day) rather than running intensity [42].

Comparing these studies on running levels of mice and rats, we notice that wild mice and laboratory mice both run for approximately 64% of a 12-hour dark period [9,41], whereas even the most active rats used by Rodnick et al. [42] ran for only 39% of the dark period. The rats used in this experiment also exhibit lower amounts of total running duration than mice, with LCR animals engaged in wheel running only 39% of the dark cycle time, and HCR 47%. The observation that rats voluntarily run at lower durations than mice suggests that rats have greater latitude for increasing the time devoted to running activity. Following this, we suggest the increase in total distance run by HCR rats results from two distinct factors. The increase in wheel-running intensity is the direct result of selection for increased endurance capacity, while the increase in wheel-running duration is a correlated response to selection for increased aerobic capacity. This hypothesis suggests that rats voluntarily exercise at a threshold or optimal level of VO_2max such that animals possessing high VO_2max (HCR) will voluntarily run at a higher intensity than animals with low VO_2max (LCR). This hypothesis has been previously proposed by Friedman et al. [39].

In order to better understand the differences in the global wheel-running activity of HCR and LCR rats, individual running bout activity was analyzed. Analyses of running bout activity are used to discern the intricacies of an animal's running behavior, and include the number of running bouts (a single period of uninterrupted running) an animal initiates per day, as well as the distance an animal covers during these bouts, and the time an animal spends engaged in a running bout [43]. Our analysis revealed significant differences between HCR and LCR rats in the number of running bouts initiated per day, as well as a trend toward differences in the speed, duration and distance run during these running bouts. Our results suggest that the increased activity levels of HCR animals are primarily due to an increase in the number of times running is initiated per day (bouts per day). The trends toward a prolonged duration of running bouts (minutes per bout), and increased intensity of running during these bouts (bout speed) also contributes to this difference in total running. The higher level of bout initiation, and the prolonged maintenance of these bouts by HCR rats results in an increase in the amount of time these animals spend running. Therefore, higher levels of bout initiation and bout duration could be a correlated response to selection for endurance capacity, while HCR animals trending towards increased running intensity during these bouts could be the direct result of selection for endurance capacity.

As discussed by Eikelboom [44], bin size (the frequency at which wheel running is collected and pooled) can affect the perceived behavior of bout running. He states that the use of a large bin size (>5 s) can result in the overestimation of an animal's running speed, and can also obscure differences that exist between groups [44]. The apparatus used in the current study recorded wheel-running data once per minute (a bin size of 1 min) which is far above the threshold suggested by

Eikelboom [44]. Regardless, we observe differences the number of bouts per day, a strong trend ($P=0.07$) in bout running speed, and a trend ($P=0.115$) in distance run per bout, and bout duration. These differences, despite our large bin size further strengthens our conclusion that HCR and LCR rats exhibit differences in their intermittent wheel-running behavior.

Voluntary activity levels are influenced by multiple internal and external factors such as hormones, body mass, and food availability (see [45,46] for review). Consequently, a correlated response to selection could result from changes in any one, or a combination of these factors. Body mass is negatively correlated with voluntary wheel-running levels in mice selected for high levels of voluntary wheel running [12]. The presence of a running wheel however, does not affect body mass in female LCR and HCR rats, which is consistent with previous observations on the effects of wheel running on body composition in female rats [47]. These observations suggest that body mass affects daily voluntary activity levels, but conversely, wheel-running activity does not alter body mass in female rats.

Although endurance capacity predicts voluntary wheel-running distance, duration, and speed in the HCR and LCR lines, individual endurance scores and voluntary wheel running are not correlated within the selected lines. These results are surprising, given the relationship between endurance capacity and wheel running between the lines, as well as the repeatability of wheel-running behavior over time within individuals (Table 3). These individual correlation values are in accordance with a Lambert et al. [48] study on wild-type rats, which demonstrated that an individual's VO_2max does not predict voluntary activity levels. One possible reason for this observation in our study is the approximately 13 weeks that separate the test for aerobic capacity from the initiation of wheel-running activity recordings. This time lapse could have resulted in small but significant changes in individuals, which obscured the correlation between these measures. Furthermore, a study by De Bono et al. [49] suggests that our method of data collection could have contributed to the lack of correlation we observe between individual levels of endurance capacity and voluntary activity. De Bono et al. [49] observed running behavior of C57BL/6 mice by collecting each revolution of running instantaneously, with no pooling of data into bins of time. This method of data collection, which eliminates misrepresentations of running behavior that result from averaging or totaling activity, revealed that an animal's average running speed was not indicative of its actual preferred running speed. The method we used to collect wheel-running activity could have misrepresented individual preferred running behavior as to obscure correlations with their individual endurance capacity scores.

Physical activity, such as voluntary wheel running, can elicit a response in plasma corticosterone levels [18,50]. Additionally, long term exercise can modulate the activity of stress hormones, resulting in decreased basal levels of corticosterone and a dampened response of corticosterone to stressful stimuli [23]. Animals from LCR and HCR populations exhibit similar resting levels of plasma corticosterone; however following 8 weeks of wheel running HCR animals express lower levels of plasma corticosterone than LCR animals. This difference results from both an increasing trend in LCR animals, and a small decreasing trend in HCR animals in plasma corticosterone concentrations. Chronic voluntary exercise results in a decrease in basal plasma corticosterone concentrations [51]. However, in this study, LCR animals exhibit a trend toward increased plasma corticosterone concentrations following running wheel access, leading to higher levels of plasma corticosterone concentrations in LCR animals than in HCR animals following 8 weeks of running wheel access. These differences suggest that either wheel running is more physiologically stressful for LCR animals, regardless of its voluntary nature, or that HCR animals potentially benefit more from this physical exercise, as increased resting plasma corticosterone concentrations have been associated with decreased health [52].

Voluntary wheel running is a motivated locomotor activity [45], and these types of behaviors are strongly influenced by striatal dopamine [53]. Drugs of abuse that stimulate striatal dopaminergic activity via DA release or by blocking DA reuptake, rapidly initiate motivated locomotor activity [54,55]. The data suggest that dopaminergic systems innervating the striatum should be affected by selection for motivated activities. Selection for endurance capacity clearly influences striatal dopaminergic activity (Fig. 6). It is an open question whether the selection regime that produces increased endurance capacity also includes selection for nigrostriatal dopaminergic elements. However, while HCR animals exhibit elevated basal striatal dopaminergic activity compared to LCR, wheel running affects dopaminergic activity in opposite directions in the two lines. HCR animals with high endurance and elevated baseline striatal dopaminergic activity exhibit a slight, but insignificant decrease in dopaminergic activity with access to running wheels. In contrast, a slight but insignificant elevation in dopaminergic activity was detected in the LCR group housed with a running wheel when compared to LCR rats without access to running wheels. The levels of dopaminergic activity between HCR and LCR selected lines did not differ when a running wheel was available. This result suggests the possibility that a specific level of dopaminergic activity may be optimal for maximizing exercise ability and may partly explain the common asymptote in wheel-running activity levels approached by both low (LCR) and high (HCR) endurance capacity selected lines (Fig. 2a).

In conclusion, selection for endurance capacity affected the global and intermittent voluntary wheel-running behavior of N-NIH rats. The quantitative nature of these measures and their associations with a multitude of other physiological traits provide an impetus to better understand the genetics behind variations observed in these traits, and how they influence one another. These animals demonstrate differences in plasma corticosterone levels, and the response of corticosterone to exercise differences that could prove significant in future experiments investigating the effect of exercise on stress systems, including corticosterone. Finally, we introduced a central dopaminergic mechanism that may be involved in the observed differences in the activity levels of these animals. Future research with these animals will examine possible differences in basal levels of behavior, hormonal responsivity and

additional neurochemical measures, and how voluntary exercise affects these traits.

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References

[1] Lynch MWB. Genetics and analysis of quantitative traits. Sunderland, MA: Sinauer Assoc., Inc.; 1998.
[2] Bouchard C, Lesage R, Lortie G, Simoneau J, Hamel P, Boulay M, et al. Aerobic performance in brothers, dizygotic and monozygotic twins. Med Sci Sports Exerc 1986;18:639–46.
[3] Simonen R, Perusse L, Rankinen T, Rice T, Rao D, Bouchard C. Familial aggregation of physical activity levels in the Quebec family study. Med Sci Sports Exerc 2002;34:1137–42.
[4] Garland T. Evolutionary physiology. Annu Rev Physiol 1994;57:579–621.
[5] Gibbs A. Laboratory selection for the comparative physiologist. J Exp Biol 1999;202:2709–18.
[6] Falconer D. Introduction to quantitative genetics. (3rd Edition). New York, NY: Longman Scientific & Technical; 1989.
[7] Harris R. Alcohol intoxication: ion channels and genetics. FASEB J 1989;3:1689–95.
[8] Koch L, Britton S. Artificial selection for intrinsic aerobic endurance running capacity in rats. Physiol Genomics 2001;5:45–52.
[9] Swallow J, Carter P, Garland Jr T. Artificial selection for increased wheel-running behavior in house mice. Behav Genet 1998;28:227–37.
[10] Garland Jr T. Selective breeding for high endurance running increases hindlimb symmetry. Evolution 2005;59:1851–4.
[11] Koteja P, Garland Jr T, Sax J, Swallow J, Carter P. Behaviour of house mice artificially selected for high levels of voluntary wheel running. Anim Behav 1999;58:1307–18.
[12] Rezende E, Chappell M, Gomes F, Malisch J, Garland Jr T. Maximal metabolic rates during voluntary exercise, forced exercise, and cold exposure in house mice selectively bred for high wheel-running. J Exp Biol 2005;208:2447–58.
[13] Rhodes J, Garland Jr T, Gammie S. Patterns of brain activity associated with variation in voluntary wheel-running behavior. Behav Neurosci 2003;117:1243–57.
[14] Swallow J, Garland Jr T, Carter P, Zhan W, Sieck G. Effects of voluntary activity and genetic selection on aerobic capacity in house mice (*Mus domesticus*). J Appl Physiol 1998;84:69–76.
[15] Dumke C, Rhodes J, Garland Jr T, Maslowski E, Swallow J, Wetter AC, et al. Genetic selection of mice for high voluntary wheel-running: effect on skeletal muscle glucose uptake. J Appl Physiol 2001;91:1289–97.
[16] Houle-Leroy P, Garland Jr T, Swallow J, Guderley H. Effects of voluntary activity and genetic selection on muscle metabolic capacities in house mice *Mus domesticus*. J Appl Physiol 2000;89:1708–17.
[17] Wisløff U, Najjar S, Ellingsen O, Haram P, Swoap S, Al-Share Q, et al. Cardiovascular risk factors emerge after artificial selection for low aerobic capacity. Science 2005;307:418–20.
[18] Coleman M, Garland Jr T, Marler CA, Newton SS, Swallow J, Carter P. Glucocorticoid response to forced exercise in laboratory house mice (*mus domesticus*). Physiol Behav 1998;63:279–85.
[19] Tharp G. The role of glucocorticoids in exercise. Med Sci Sports Exerc 1975;7:6–11.
[20] Boyle M, Kolber B, Vogt S, Wozniak D, Muglia L. Forebrain glucocorticoid receptors modulate anxiety-associated locomotor activation and adrenal responsiveness. J Neurosci 2006;26:1971–8.
[21] Sauro M, Jorgensen R, Pedlow C. Stress, glucocorticoids, and memory: a meta-analytic review. Stress 2003;6:235–45.
[22] Girard I, Garland Jr T. Plasma corticosterone response to acute and chronic voluntary exercise in female house mice. J Appl Physiol 2002;92:1553–61.
[23] Droste S, Gesing A, Ulbricht S, Muller MB, Lindthorst A, Reul J. Effects of long-term voluntary exercise on the mouse hypothalamic-pituitary–adrenocortical axis. Endocrinology 2003;144:3012–23.
[24] Dishman R, Berthoud H, Booth F, Cotman C, Edgerton V, Fleshner M, et al. Neurobiology of exercise. Obesity 2006;14:345–56.
[25] Ingram D. Age-related decline in physical activity: generalization to nonhumans. Med Sci Sports Exerc 2000;32:1623–9.
[26] Brosse A, Sheets E, Lett H, Blumenthal J. Exercise and the treatment of clinical depression in adults. Sports Med 2002;32:741–60.
[27] Cotman C, Engesser-Cesar C. Exercise enhances and protects brain function. Exerc Sport Sci Rev 2002;30:75–9.
[28] Gilbert C. Optimal physical performance in athletes: key roles of dopamine in a specific neurotransmitter/hormonal mechanism. Mech Ageing Dev 1995;84:83–102.
[29] Palkovits M, Brownstein M. Maps and guide to microdissection of the rat brain. Amsterdam: Elsevier; 1988. Netherlands.
[30] Renner K, Luine V. Analysis of temporal and dose-dependent effects of estrogen on monoamines in brain nuclei. Brain Res 1986;366:64–71.
[31] Renner K, Luine V. Determination of monoamines in brain nuclei by high performance liquid chromatography with electrochemical detection: young vs. middle-aged rats. Life Sci 1984;34:2193–9.
[32] McKay L, Bradberry C, Oke A. Ascorbic acid oxidase speeds up analysis for catecholamines, indoleamines and their metabolites in brain tissue using HPLC-EC. J Chromatogr 1984;311:167–9.
[33] Bradford M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.
[34] Lerman I, Harrison B, Freeman K, Hewett T, Allen D, Robbins J, et al. Genetic variability in forced and voluntary endurance exercise performance in seven inbred mouse strains. J Appl Physiol 2002;92:2245–55.
[35] Lightfoot J, Turner M, Daves M, Vordermark A, Kleeberger S. Genetic influence on daily wheel-running activity level. Physiol Genomics 2004;19:270–7.
[36] Turner M, Kleeberger S, Lightfoot J. Influence of genetic background on daily running-wheel activity differs with aging. Physiol Genomics 2005;22:76–85.
[37] Koch L, Britton S, Barbato J, Rodenbaugh D, DiCarlo S. Phenotypic differences in cardiovascular regulation in inbred rat models of aerobic capacity. Physiol Genomics 1999;1:63–9.
[38] Lightfoot J, Turner M, Debate K, Kleeberger S. Interstrain variation in murine aerobic capacity. Med Sci Sports Exerc 2001;33:2053–7.
[39] Friedman W, Garland Jr T, Dohm M. Individual variation in locomotor behavior and maximal oxygen consumption in mice. Physiol Behav 1992;52:97–104.
[40] Desan P, Silbert LH, Maier SF. Long-term effects of inescapable stress on daily running activity and antagonism by desipramine. Biochem Behav 1998;1:21–9.
[41] Dohm M, Richardson C, Garland Jr T. Exercise physiology of wild and random-bred laboratory house mice and their reciprocal hybrids. Am J Physiol, Regul Integr Comp Physiol 1994;267:R1098–110.

[42] Rodnick KJ, Reaven GM, Haskell W, Sims CR, Mondon CE. Variations in running activity and enzymatic adaptations in voluntary running rats. J Appl Physiol 1989;77:1250–7.
[43] Eikelboom R, Mills R. A microanalysis of wheel running in male and female rats. Physiol Behav 1988;43:625–30.
[44] Eikelboom R. Bins, bouts and wheel-running speed. Anim Behav 2001;71:779–81.
[45] Sherwin C. Voluntary wheel running: a review and novel interpretation. Anim Behav 1998;56:11–27.
[46] Rhodes J, Gammie S, Garland Jr T. Neurobiology of mice selected for high voluntary wheel-running activity. Integr Comp Biol 2005;45:438–55.
[47] Cortright R, Chandler M, Lemon P, DiCarlo S. Daily exercise reduces fat, protein and body mass but not in female rats. Physiol Behav 1997;92: 105–11.
[48] Lambert M, Van Zyl C, Jaunky R, Lambert E, Noakes T. Tests of running performance do not predict subsequent spontaneous running in rats. Physiol Behav 1996;60:171–6.
[49] De Bono JP, Adlam D, Paterson D, Channon K. Novel quantitative phenotypes of exercise training in mouse models. Am J Physiol, Regul Integr Comp Physiol 2006;290:R926–34.
[50] Devenport L, Doughty D, Heiberger B, Burton D, Brown R, Stith R. Exercise endurance in rats: roles of type I and II corticosteroid receptors. Physiol Behav 1993;53:1171–5.
[51] Viru M, Litvinova L, Smirnova T, Viru A. Glucocorticoids in metabolic controls during exercise: glycogen metabolism. J Sports Med Phys Fitness 1994;34:377–82.
[52] Wingfield J, Hunt K, Breuner C, Dunlap K, Fowler G, Freed L, et al. Behavioral approaches to conservation in the wild. Cambridge, UK: Cambridge University Press; 1997.
[53] Ouchi Y, Yoshikawa E, Futatsubashi M, Okada H, Torizuka T, Sakamoto M. Effect of simple motor performance on regional dopamine release in the striatum in Parkinson disease patients and healthy subjects: a Positron Emission Tomography Study. 22: 746–752 (2002).
[54] Ikemoto S. Ventral striatal anatomy of locomotor activity induced by cocaine, D-amphetamine, dopamine and D1/D2 agonists. Neuroscience 2002;113:939–55 Neuroscience 113: 939–955.
[55] Joyce E, Koob G. Amphetamine-, scopolamine- and caffeine-induced locomotor activity following 6-hydroxydopamine lesions of the mesolimbic dopamine system. Psychopharmacology 1981;73:311–3.