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Serotonin-mediated central fatigue underlies increased endurance capacity in mice from lines selectively bred for high voluntary wheel running

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HIGHLIGHTS

- Male mice were tested for forced treadmill endurance and voluntary wheel running.
- A serotonin (5-HT_{1A}) agonist or antagonist (varying doses) was administered.
- The effect of each drug on endurance depended on genetic background.
- Effects on wheel running differed from effects on treadmill endurance.
- Results point to genetically determined control of endurance and fatigue.

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ABSTRACT

Serotonin (5-hydroxytryptamine; 5-HT) is implicated in central fatigue, and 5-HT_{1A} pharmaceuticals are known to influence locomotor endurance in both rodents and humans. We studied the effects of a 5-HT_{1A} agonist and antagonist on both forced and voluntary exercise in the same set of mice. This cohort of mice was taken from 4 replicate lines of mice that have been selectively bred for high levels of voluntary wheel running (HR) as compared with 4 non-selected control (C) lines. HR mice run voluntarily on wheels about 3× as many revolutions per day as compared with C, and have greater endurance during forced treadmill exercise. We hypothesized that drugs targeting serotonin receptors would have differential effects on locomotor behavior of HR and C mice. Subcutaneous injections of a 5-HT_{1A} antagonist (WAY-100,635), a combination of 5-HT_{1A} agonist and a 5-$HT_{1A/1B}$ partial agonist (8-OH-DPAT + pindolol), or physiological saline were given to separate groups of male mice before the start of each of three treadmill trials. The same manipulations were used later during voluntary wheel running on three separate nights. WAY-100,635 decreased treadmill endurance in HR but not C mice (dose by linetype interaction, $P = 0.0014$). 8-OH-DPAT + pindolol affected treadmill endurance ($P < 0.0001$) in a dose-dependent manner, with no dose by linetype interaction. Wheel running was reduced in HR but not C mice at the highest dose of 8-OH-DPAT + pindolol (dose by linetype, $P = 0.0221$), but was not affected by WAY-100,635 treatment. These results provide further evidence that serotonin signaling is an important determinant of performance during both forced and voluntary exercise. Although the elevated wheel running of HR mice does not appear related to alterations in serotonin signaling, their enhanced endurance capacity does. More generally, our results indicate that both forced and voluntary exercise can be affected by an intervention that acts (primarily) centrally.

1. Introduction

When maximal effort expended voluntarily is less than what can be achieved through induced effort (such as by electrical stimulation of a motor nerve), then it can be said that the motor output is limited centrally [1]. Central fatigue potentially limits the performance of an organism to less than the level that might be predicted by classical models of physiological maxima, and can be caused by reduced drive to motor neurons from the central nervous system. The monoamine hypothesis of central fatigue posits that the balance of neurotransmitters (including serotonin [5-hydroxytryptamine; 5-HT], dopamine [DA], and norepinephrine [NE]) influences thermoregulation, motor impulses, and sensations of fatigue, thus contributing to central control of the duration and intensity of exercise [reviewed in 2]. Of these neurotransmitters, serotonin has been the most studied with specific

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reference to central fatigue, although the effect of dopamine receptor agonists and transport inhibitors during exercise is well established [3,4]. The predictive factor in 5-HT-generated central fatigue may not be 5-HT concentration alone, but the ratio of 5-HT:DA in such brain regions as the striatum [2]. The relevance of that ratio may be because 5-HT appears to indirectly and directly regulate dopamine at multiple levels [reviewed in 5].

Although the results of pharmaceutical 5-HT manipulations in human athletes have been inconsistent [2], pharmacological studies with rodents support the monoamine hypothesis of central fatigue. Pharmacological manipulations of rat 5-HT receptors [6,7] and central injection of 5-HT precursor L-Tryptophan [8,9] are sufficient to influence the performance of forced exercise at the whole-animal level. Administration of the 5-HT_3 agonist quipazine dimaleate decreased time to exhaustion in rats running on a motorized treadmill (with physical prodding for motivation), whereas 5-HT_2 antagonist LY 53857 increased time to exhaustion [6]. At exhaustion, agonist-treated animals had higher plasma glucose, liver glycogen, and muscle glycogen than vehicle-treated animals suggesting that they fatigued before they depleted energy stores. In a similar study with rats, agonists of 5-HT_{1A} receptors increased endurance, whereas an antagonist of 5-HT_{1A} receptors caused early exhaustion [7]. Numerous studies of rats have implicated the release of 5-HT in the onset of fatigue [10–12]. 5-HT is released to multiple brain regions, and specific downstream effects can differ depending on brain region and receptor subtype [the functions of 5-HT in the brain are reviewed in 13,14–16]. 5-HT receptors in the spinal cord also have important functions related to exercise. 5-HT receptors can be found in the dorsal horn of the spinal cord, and affect the excitability of motor neurons [17–19]. Despite its apparent relation to fatigue, 5-HT has been shown to promote excitability in motor neurons in small concentrations [20,21], but high concentrations spill over onto initial axon segments, and can inhibit action potentials in motor neurons [22,23]. Finally, many authors assert that the primary connection between 5-HT and fatigue is through its effects on thermoregulation [e.g., 24].

The relationship between maximal exercise capacity and voluntary locomotor activity is unclear. The measures of treadmill endurance and voluntary wheel running are not correlated in rodent studies of individual variation [25,26] or strain variation [27,28]. However, selective breeding for high voluntary wheel running in mice has resulted in increased treadmill endurance as compared with non-selected controls [29], and bidirectional selective breeding for treadmill endurance (one line selected for high endurance vs. one line selected for low endurance) has resulted in differential voluntary wheel running (rats from the high-endurance line run more on wheels than those from the low-endurance line) [30]. Rodents with wheel access can effectively train themselves to perform better at forced treadmill tasks [31], and can have altered 5-HT receptor mRNA expression, including a reduction in 5-HT_{1A} mRNA after 6 weeks of wheel access [32].

Mice from lines that have been selectively bred for high voluntary wheel running (high-runner or HR) run about three times as many wheel revolutions per day as controls (C). The absolute number of revolutions run per day reached a plateau around generation 16–28 depending on line and sex despite continued selective breeding [33]. The difference in total wheel revolutions is caused primarily by an increase in average speed of running, rather than an increase in the amount of time spent running [34], although male HR mice do run for significantly longer per night than male controls [35]. A number of correlated responses to selection have been described in the HR mice, including increases in two whole-organism measurements of exercise capacity, forced treadmill endurance [29] and maximal exercise-induced oxygen consumption [36–38]. HR differ from C mice in circulating hormone concentrations, having higher corticosterone [39–42], lower leptin [43], and higher adiponectin [44] as compared with C mice (depending on sex and age). HR mice have larger brains, specifically the midbrains [45], and have widespread, differential (compared to C mice) elevation of brain activity as measured by immediate early genes during exercise and when prevented from exercising [46,47]. HR mice often have different reactions to environmental conditions and pharmaceuticals. Ritalin administration decreased wheel-running in HR mice while increasing wheel-running in C mice [48]. Administration of a type 1 cannabinoid receptor (CB_1) agonist and antagonist had sex-specific differential effects on HR vs. C mice [49,50]. Administration of leptin increased wheel running only in HR mice [51].

The purpose of the present study was to determine if a 5-HT pharmaceutical manipulation would differentially affect endurance in mice with an innately high endurance capacity as compared with "standard" mice, and to shed light on how endurance can evolve with respect to central fatigue. The high-runner mouse model is particularly well suited for study of the evolution of central fatigue because (1) the HR mice have evolved greater endurance than their non-selected control counterparts [29], (2) they have many described neurobiological differences, including differential responses to various pharmaceuticals [46,48–50,52] and leptin [51], (3) they are well studied in terms of physiological adaptations to high activity as well as patterns of behavior, and (4) methodologically, there is an established paradigm for studying these mice as they exercise voluntarily, pacing themselves through an entire night of high-speed wheel running. Furthermore, the model contains 4 replicate selected lines and 4 non-selected control lines, which allows considerable power of inference as compared with most vertebrate selection experiments [53,54]. To our knowledge, no experiment has used pharmacological manipulations with voluntary and forced exercise on the same set of animals. Because wheel running is self-paced, it can provide unique insights into the chemical basis of central fatigue.

2. Methods

2.1. Animals

We used male mice (*Mus domesticus*) from post-selection generation 65 of the ongoing High-Runner selection experiment [55,56]. In brief, the experiment started with outbred Hsd:ICR stock (Harlan Sprague Dawley, Indianapolis, Indiana, USA), from which four replicate lines were selectively bred for high voluntary wheel running on days 5 and 6 of a 6-day period of wheel access (High Runner or HR lines) while four other lines were bred without respect to running (Control or C lines). Mice are weighed, toe-clipped, and weaned at 21 days of age, and housed 4 per cage by sex and line. At 6–8 weeks of age, mice are individually housed in cages with wheel access (wheel circumference 1.12 m) for 6 days. Wheel revolutions are recorded in 1-minute intervals by a photocell counter attached to the wheel and compiled via customized software (photocells and software from San Diego Instruments, San Diego, California, USA). Breeders in the HR lines are identified as the mice that run the largest number of wheel revolutions on days 5 and 6 of the trial, whereas C mice are bred randomly, except that no sibling pairings are allowed in any line. In all selection generations and experiments, mice are kept on a 12-hour light/dark cycle with ad lib food and water.

Ninety-six male mice were obtained at weaning, with 6 mice from each of the 8 lines making 48 mice for each of the two treatment (drug) types. Where possible, no siblings were placed within the same treatment groups. Mice were housed 4 per cage from the time of weaning, through the treadmill testing, then housed singly during wheel testing. Throughout the experiment, mice were kept on a 12-hour light/dark cycle (lights on 06:00 h and lights off 18:00 h) with ad lib food and water. Assignment to each drug type, the order or dose treatments, the order of treadmill testing, and the placement on wheels were randomized, and experimenters were blind to line, linetype (HR or C), and dose where appropriate. Treadmill trials occurred when mice were approximately 6–8 weeks of age, and wheel testing occurred when mice were 8–11 weeks of age. Animal procedures were approved by the University Institutional Animal Care and Use Committee (UCR

IACUC AUP# A-20110014), and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Drug protocol

Half of all of the mice were designated to receive subcutaneous injections of 5-HT_{1A} agonist 8-Hydroxy-2-dipropylaminotetralin hydrobromide (8-OH-DPAT) + 5-$HT_{1A/1B}$ partial agonist 1-(1H-Indol-4-yloxy)-3-[(1-methylethyl)amino]-2-propanol (pindolol), and half received a 5-HT_{1A} antagonist *N*-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexanecarboxamide maleate (WAY-100,635; all drugs obtained from Tocris Bioscience, Bristol, UK). The mice received a subcutaneous injection of the designated drug 15 min prior to the start of each of three treadmill trials, and in the middle of voluntary wheel running on three separate nights. There were more than two weeks of rest between treadmill and wheel testing, and within each set of tests, 2 days of rest between repeated injections.

8-OH-DPAT has previously been shown to increase endurance and WAY-100,635 decreased endurance measured during forced running on a motorized drum in rats [7]. 8-OH-DPAT is a standard drug used in studying the function of the 5-HT_{1A} receptor, but also has slight affinity for the 5-HT_7 receptor and may also act as a 5-HT reuptake inhibitor [57]. 8-OH-DPAT was previously shown to increase endurance in low doses, but decrease endurance at high doses [7], but with the addition of 5-$HT_{1A/1B}$ antagonist and partial β_3 agonist pindolol, the biphasic effect disappeared and the endurance continued to increase with increasing doses of 8-OH-DPAT. For this reason, pindolol was added to 8-OH-DPAT. WAY-100,635 has also been a standard drug used in studying the function of the 5-HT_{1A} receptor, but it was discovered that it also is a potent D_4 agonist [58], and the behavioral effects of WAY-100,635 as a D_4 agonist have not been well defined [but see 59,60]. Systemic injection is perhaps not ideal for studying the manipulation of 5-HT receptors, given that the 5-HT receptors, including 5-HT_{1A}, are widespread and it is possible that the drugs have unintended effects beyond the central nervous system. However, in order to test our specific hypothesis regarding the effects of selective breeding, a large number of measurements from a large number of animals were required, making more targeted techniques such as central injection unfeasible.

Each drug was prepared at a constant concentration, and injection volumes were determined for each mouse based on body mass, ranging from 0.27–0.51 ml for the 8-OH-DPAT + pindolol group and 0.17–0.29 ml for the WAY-100,635 group. For 8-OH-DPAT, the dose was 0.2 mg/kg body weight of 8-OH-DPAT and 0.8 mg/kg of pindolol for the low dose. The high dose was 2.0 mg/kg 8-OH-DPAT and 8.0 mg/kg of pindolol. For WAY-100,635, the dose was 35 μg/kg for the low dose and 350 μg/kg for the high dose. These doses approximate the cited study of rats [7], while accounting for the difference in average body mass between our mice (about 32 g) and the rats in the cited study (about 300 g), and assuming an allometric relationship of metabolic rate with exponent = 0.75 [61]. All drugs were diluted with physiological saline, with pindolol being first dissolved in a very small volume of glacial acetic acid to aid dissolution, following Ahlenius [7]. Thus, saline was the vehicle for both drugs, and the appropriate amount of saline was also used for a control injection. However, we did not want to cause confusion in the figures because our non-selected mice are called "control" mice. For this reason, we have termed the "control" dose as the "vehicle" dose (short for "vehicle only").

2.3. Endurance testing protocol

Mouse endurance was assessed using a graded treadmill endurance test modified from previously published protocols [27,29,62]. Prior to the start of exhaustive exercise trials, mice were trained on the treadmill for 15 min on each of three consecutive days at 10 meters/minute (m/min), 12 m/min, and 14 m/min, respectively. During testing, mice began at a treadmill speed of 14 m/min and speed was increased 1.5 m/min every 2 min until the mouse was unable to continue running at the belt speed (Fig. 1). For both training and testing, the treadmill was at a 25° incline, and a stimulation grid with adjustable-amperage (0–12 mAmp) at the end of the treadmill provided motivation. Mice were judged to be exhausted when they showed an inability to maintain speed and remained on the stimulation grid for three consecutive seconds. A single judge, who was blind to linetype and treatment, made this determination for every trial in the study. Mice completed three exhaustive exercise bouts each (with one day of rest between bouts), and received one injection per bout (dose order randomized); a vehicle injection, a low dose injection, and a high dose injection. An individual mouse thus received only two injections of one drug (either 8-OH-DPAT + pindolol or WAY-100,635) during treadmill testing, with either 2 or 4 days between the low dose and high dose, depending on when the vehicle was administered.

Endurance was measured as time to exhaustion (TTE), distance traveled (a product of TTE and speed, which increased as the trial went on, Fig. 1), and vertical work performed, which is equal to the product of vertical distance (total distance multiplied by sin(25°) in meters) and the mass of the mouse in kg [63]. Each of those measurements are highly related to one another, but differ enough that we predicted that their analyses might give different results.

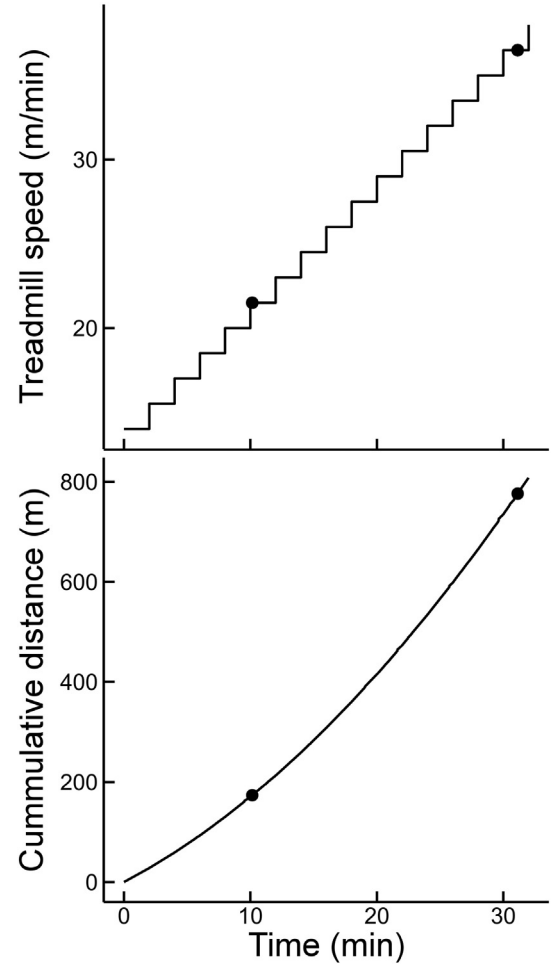

Fig. 1. Treadmill speeds and cumulative distance using a graded treadmill endurance test (modified from previously published protocols: [27,29,62]). Top panel: Mice began at a treadmill speed of 14 m/min and speed was increased 1.5 m/min every 2 min until the mouse was unable to continue running. Bottom panel: Cumulative distance run in relation to time spent running. In each panel, the points indicate the maximum and minimum values observed in this study (minima: time = 10:09 (mm:ss), treadmill speed at exhaustion: 21 m/min, distance: 173.22 m; maxima: 31:08 (mm:ss), speed: 36.5 m/min, distance: 776.31 m; treatments combined).

The testing protocol used here matches the study in which it was originally discovered that HR mice have greater endurance than C mice, and moreover in which we provided physiological evidence (blood lactate and glucose levels) indicating that it truly caused exhaustion in both HR and C mice [29]. As our specific hypothesis was related to the endurance difference between HR and C mice, these methods were most appropriate.

2.4. Wheel running protocol

Following the treadmill trials, mice were housed individually in cages with wheel access. Wheel running reached a plateau after 14 days, at which point pharmaceutical manipulations began. Mice retained their treatment designations from the treadmill trials (each mouse received the same drug for both the treadmill and wheel-running trials). Again, there were three injections (with two days of rest between injections); a vehicle injection, a low dose injection, and a high dose injection. Thus, an individual mouse received two injections of one drug (either 8-OH-DPAT + pindolol or WAY-100,635) during wheel running (in addition to the two injections given ~2 weeks earlier during treadmill testing), with either 3 or 6 days between the low and high dose, depending on when the vehicle was administered. Injections occurred between 2 and 3 h after the onset of the dark period (Fig. 2). Mice were removed from the wheels under red light, injected, and returned to their cages. This took <5 min in all cases, and data from the 10 min before and 10 min after the time that the mice were injected were discarded to avoid any artifacts of handling. Wheel revolutions were counted automatically in 1-minute bins by a photocell counter attached to a computer running an automated program. The general protocols above follow several previous pharmacology studies of HR mice [e.g. 48,49,50]. The first 10 min after injection were excluded to avoid handling effects, and the first and second hours following that period were used in separate analyses (one analysis for 10–70 and another for 70–130 min post-injection).

2.5. Statistical analysis

Statistical analyses were performed using SAS PROC MIXED. The primary factors of analysis were linetype (HR vs. C) and dose, and replicate line was used as a random effect nested within linetype. Individual was the factor for repeated measures. The statistical interaction dose by linetype was of prime interest because, where significant, it indicates a differential response of the HR and control lines to the drug doses. Wheel freeness (a measure of how easy it is to turn each wheel) was measured before and after the wheel trials and temporarily included as a covariate in statistical analyses, as was age. Age and wheel freeness were not significant predictors of wheel running, and were thus left out of the final model.

The mini-muscle phenotype, caused by a Mendelian recessive allele that was present at a low frequency of about 7% in the original base population and characterized by a 50% mass reduction in the triceps surae and total hindlimb muscle mass (in homozygotes), has increased in one HR line and eventually become fixed in another [64,65]. In the one HR line that contains both genotypes, triceps surae were dissected and weighed to determine mini-muscle status, and then mini-muscle status for those mice as well as mice within the fixed mini-muscle line was considered as a factor for the 8-OH-DPAT treatment group, but not the WAY-100,635 treatment group, in which mini-muscle was

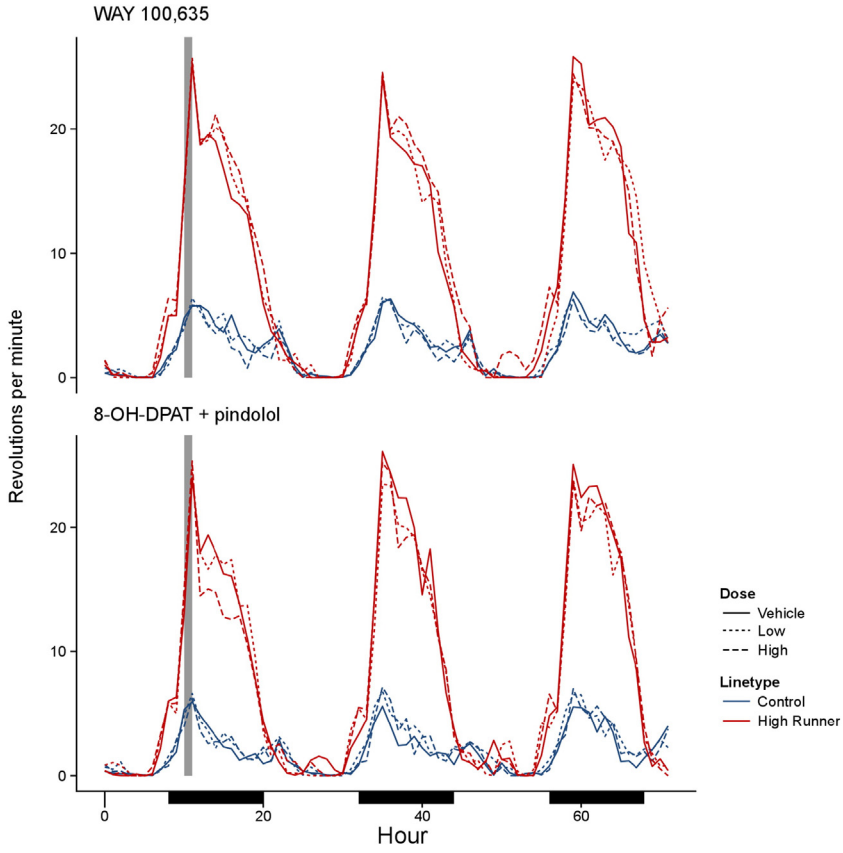

Fig. 2. Mean revolutions per minute (binned hourly) for linetype-dose groups over three consecutive days of voluntary wheel running. Aside from an acute reduction in wheel running for mice treated with the high dose of 8-OH-DPAT, the circadian pattern of wheel running was unaffected by the drugs. In the figure, hour is hours from the start of the test on the injection day, such that time 0 represents the beginning of the wheel running period on the day that the mouse was injected. Injections began two hours after the start of the scotophase. The injection period marked in light gray. The combined time to inject all mice was less than one hour. During that hour each mouse received a subcutaneous injection of a vehicle, a low dose, or high dose of either WAY 100,635 or 8-OH-DPAT + pindolol. Mice were kept on a 12:12 light:dark cycle. The dark phase began at 18:00 and the light phase began at 6:00. Dark hours are indicated by black bars on the axis.

fully confounded with line. The inclusion of mini-muscle in the model did not substantially change the findings, and is not included in the results presented here.

3. Results

WAY-100,635 differentially decreased endurance in the treadmill trial in HR as compared to C mice (dose by linetype interaction, P = 0.0020, Table 1). HR mice exhausted more slowly (were able to run longer) than C mice while under the vehicle treatment, but exhausted at nearly the same point at either the high or low dose of the drug (Fig. 3). Results were very similar for distance run to exhaustion and total work performed (see Table 1).

Time to exhaustion during the treadmill trial was significantly affected by dose in the 8-OH-DPAT + pindolol treatment ($F_{(2, 12)}$ = 34.57, P = <0.0001, Table 1), but not by linetype or the dose-by-linetype interaction (P = 0.1422, P = 0.2227, respectively). Visually, it appears that the low dose marginally increased, whereas the high dose decreased endurance in both linetypes by a roughly equal proportion (Fig. 3), but this apparent low-dose effect was not statistically significant (difference of least squares means P = 0.0566).

In all doses and drug combinations, HR mice ran significantly more wheel revolutions than C mice. For the first hour after injection, wheel running was significantly and differentially affected by 8-OH-DPAT + pindolol treatment (first hour dose by linetype, P = 0.0226, Table 2), and similarly to the treadmill results, the effect was most noticeable at the high dose, at which voluntary wheel running was dramatically reduced (Fig. 4). The effect seemed to be more strongly related to a reduction in speed (first hour $F_{(2, 12)}$ = 5.96, P = 0.0160 for RPM versus $F_{(2, 12)}$ = 2.41, P = 0.1321 for active minutes). In the second hour after injection, the effect mostly disappeared (Table 3, Figs. 4). Wheel running was not significantly affected by WAY-100,635 treatment (first hour dose P = 0.8902, Tables 2, 3, Figs. 4).

4. Discussion

We found that a $5HT_{1A}$ antagonist (WAY-100,635) differentially decreased treadmill endurance performance in mice that have evolved high endurance capacity due to selective breeding for voluntary exercise, but did not affect endurance of their non-selected control lines. Perhaps surprisingly, a reduction in endurance capacity of HR mice was not paralleled by a reduction in voluntary wheel running. We did find that voluntary wheel running of HR mice was differentially affected by a serotonin agonist (8-OH-DPAT + pindolol) 10–70 min post-injection, which is the first evidence that serotonin signaling accounts for any of the behavioral differences between HR and C mice. Overall, these results demonstrate that pharmacological interventions acting on central nervous system 5-HT receptors can influence endurance and voluntary exercise in different ways, and they shed light on the microevolution of locomotor behavior.

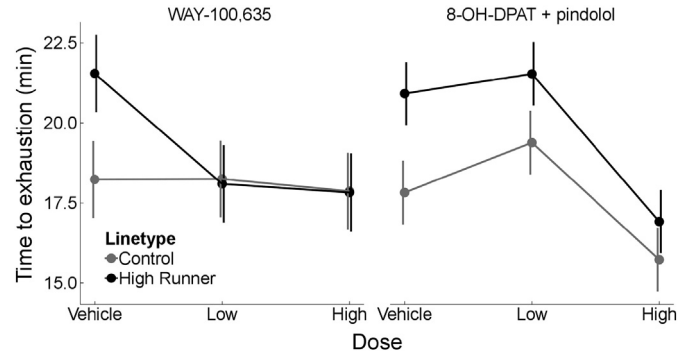

Fig. 3. Time to exhaustion (min) under a graded-exercise protocol on an inclined treadmill (25° angle) with manual and electrical stimulation for HR and C mice treated with vehicle (saline), low or high doses of either WAY-100,635 (serotonin antagonist) or 8-OH-DPAT + pindolol (serotonin agonist). For both drugs, the interaction between linetype and drug is highly statistically significant (see Table 1). Values are least square means ± associated standard errors from repeated-measures analysis in SAS Procedure Mixed, as reported in Table 1.

4.1. Relevance for the high runner selection experiment

This is the first study to provide evidence that alterations in serotonin signaling account for some of the known behavioral or performance differences between HR and C mice. For mice treated with WAY-100,635, endurance (measured as running time to exhaustion) decreased only in HR, and not C mice (Fig. 3), which surprisingly indicates

Table 1
Treadmill endurance-running performance. Values are least square means ± associated standard errors from repeated-measures analysis in SAS Procedure Mixed. Different sets of mice were used for the two drugs, with sample size of 49 mice per drug. These values are after accounting for increases in performance with trial number (results not shown). The three alternative measures of exhaustion (time, distance, and vertical work) do not meaningfully change the results or interpretation. P values < 0.05 are in bold font.

Variable and drug	Dose			Dose effect	Linetype effect	Dose by linetype effect
	Vehicle	Low	High			
Time to exhaustion (min)						
WAY-100,635						
C	18.2 ± 1.2	18.3 ± 1.2	17.9 ± 1.2	F (DF) = 13.50 (2, 12)	F (DF) = 0.40 (1, 6)	F (DF) = 10.91 (2, 12)
HR	21.5 ± 1.2	18.1 ± 1.2	17.8 ± 1.2	**P = 0.0008**	P = 0.5482	**P = 0.0020**
8-OH-DPAT + pindolol						
C	17.8 ± 1	19.4 ± 1.0	15.7 ± 0.1.0	F (DF) = 34.57 (2, 12)	F (DF) = 2.85 (1, 6)	F (DF) = 1.71 (2, 12)
HR	20.9 ± 1	21.5 ± 1.0	16.9 ± 1.0	**P < 0.0001**	P = 0.1422	P = 0.2227
Distance to exhaustion (m)						
WAY-100,635						
C	369.3 ± 34.0	370.5 ± 33.8	360.2 ± 33.9	F (DF) = 14.01 (2, 12)	F (DF) = 0.46 (1, 6)	F (DF) = 11.77 (2, 12)
HR	464.8 ± 34.1	369.0 ± 34.2	360.1 ± 34.5	**P = 0.0007**	P = 0.5247	**P = 0.0015**
8-OH-DPAT + pindolol						
C	358.0 ± 28.4	399.2 ± 28.4	304.2 ± 28.4	F (DF) = 31.55 (2, 12)	F (DF) = 3.06 (1, 6)	F (DF) = 2.04 (2, 12)
HR	448.9 ± 28.1	466.2 ± 28.2	336.1 ± 28.1	**P < 0.0001**	P = 0.1310	P = 0.1727
Work to exhaustion (joules)						
WAY-100,635						
C	5.2 ± 0.4	5.1 ± 0.4	5.1 ± 0.4	F (DF) = 11.36 (2, 12)	F (DF) = 0.05 (1, 6)	F (DF) = 9.20 (2, 12)
HR	5.9 ± 0.4	4.6 ± 0.5	4.5 ± 0.5	**P = 0.0017**	P = 0.8350	**P = 0.0038**
8-OH-DPAT + pindolol						
C	5.1 ± 0.4	5.7 ± 0.4	4.3 ± 0.4	F (DF) = 30.88 (2, 12)	F (DF) = 0.45 (1, 6)	F (DF) = 1.32 (2, 12)
HR	5.7 ± 0.3	5.9 ± 0.4	4.3 ± 0.3	**P < 0.0001**	P = 0.5261	*P* = 0.3026

Table 2
Voluntary wheel running over the 10–70 min post-injection. Values are least square means ± associated standard errors from repeated-measures analysis in SAS Procedure Mixed. Different sets of mice were used for the two drugs, with sample size of 49 mice per drug. A variable coding for test night (1–3) were also included in the analyses, and measure of wheel freeness was considered as a covariate, but was not a significant predictor and was ultimately not included (results not shown). P values < 0.05 are in bold font.

Variable and drug	Dose			Dose effect	Line type effect	Dose by linetype effect
	Vehicle	Low	High			
Total revolutions per 10 min						
WAY-100,635						
C	41.9 ± 21.4	46.9 ± 21.4	33.1 ± 21.4	F (DF) = 0.12 (2, 12)	F (DF) = 32.97 (1, 6)	F (DF) = 0.71 (2, 12)
HR	191.4 ± 21.6	196.7 ± 21.6	206.6 ± 21.6	P = 0.8902	**P = 0.0012**	P = 0.5126
8-OH-DPAT + pindolol						
C	33.3 ± 19.8	24.0 ± 19.8	24.9 ± 19.8	F (DF) = 5.35 (2, 12)	F (DF) = 26.51 (1, 6)	F (DF) = 5.29 (2, 12)
HR	174.7 ± 19.7	181.2 ± 19.7	136.3 ± 19.7	**P = 0.0218**	**P = 0.0021**	**P = 0.0226**
Active minutes						
WAY-100,635						
C	3.7 ± 0.9	4.1 ± 0.9	3.2 ± 0.9	F (DF) = 0.66 (2, 12)	F (DF) = 13.19 (1, 6)	F (DF) = 1.44 (2, 12)
HR	7.6 ± 0.9	7.9 ± 0.9	8.3 ± 0.9	P = 0.5368	**P = 0.0109**	P = 0.2749
8-OH-DPAT + pindolol						
C	3.1 ± 0.6	2.4 ± 0.6	2.6 ± 0.6	F (DF) = 2.65 (2, 12)	F (DF) = 32.47 (1, 6)	F (DF) = 2.41 (2, 12)
HR	7.8 ± 0.6	7.8 ± 0.6	6.5 ± 0.6	P = 0.1114	**P = 0.0013**	P = 0.1321
Average wheel speed (RPM)						
WAY-100,635						
C	5.5 ± 2.0	5.9 ± 2.0	4.5 ± 2.0	F (DF) = 0.13 (2, 12)	F (DF) = 39.71 (1, 6)	F (DF) = 0.87 (2, 12)
HR	20.7 ± 2.0	21.2 ± 2.0	22.3 ± 2.0	P = 0.8789	P = 0.0007	P = 0.4433
8-OH-DPAT + pindolol						
C	4.9 ± 2.0	3.8 ± 2.0	3.4 ± 2.0	F (DF) = 7.26 (2, 12)	F (DF) = 31.50 (1, 6)	F (DF) = 5.96 (2, 12)
HR	19.5 ± 2.0	20.9 ± 2.0	15.9 ± 2.0	**P = 0.0086**	**P = 0.0014**	**P = 0.0160**

that the mechanism(s) which confers increased treadmill endurance to HR mice can be completely eliminated by this drug. We do not suggest that the difference in endurance between linetypes is attributable only to differences in 5-HT_{1A} receptors, or even more generally neurobiological differences. HR mice have many physical qualities which might make them more capable endurance runners [37,66]. However, the results here are remarkable in that the WAY-100,635-treated HR mice had virtually the same endurance as C mice (Fig. 3).

The high dose of 8-OH-DPAT had a differential effect on wheel running 10–70 min post-injection, with HR mice showing a greater reduction than C mice. This type of interactive effect has been found in previous studies of wheel running in HR and C mice. Drugs targeting endocannabinoid receptors [CB1 agonist WIN 55,212-2 and antagonist rimonabant, 49,50], dopamine receptors [non-selective dopamine agonist Ritalin, D1-like agonist SCH 23390, 48], and dopamine transporters [DAT; cocaine and GBR 12909, 52] have all differentially decreased wheel running in HR versus C mice. Cocaine has similar interactive effects on HR and C mice, but this drug acts on DAT in addition to the serotonin transporter [52]. However, the SERT inhibitor fluoxetine reduced wheel running in HR and C mice equally, indicating that the differential effect of cocaine was most likely caused by its actions on DAT [52]. Therefore, the present study provides the first strong evidence that serotonin (or possibly D_2-like receptors) may account for some of the behavioral differences between HR and C mice.

It is worth mentioning that some part of the consistent finding of drug by linetype interactions in pharmacological manipulations may be attributable to differences in pharmacokinetics between HR and C mice. HR mice are smaller [67], which is generally associated with higher mass-specific metabolic rates, and also have higher metabolic rates when running on wheels due to their greater speed of wheel running [68].

High Runner mice have innately higher endurance during forced treadmill exercise [29], likely owing in part to physical and physiological traits, such as HR mice having larger hearts and higher maximal aerobic capacity [37,66]. In addition, HR and C mice differ in muscle properties. HR lines tend to have reduced triceps surae muscle masses (beyond mini-muscle effects - see following) [69], and we have demonstrated differences between HR and C lines (beyond mini-muscle effects) for fiber types in the tibialis anterior muscle [70]. Further, HR lines show increased adaptive plasticity in gastrocnemius GLUT-4 concentrations [71]. When housed without wheel access, no differences in gastrocnemius GLUT-4 were observed. After 5 days with wheels, all mice showed elevated GLUT-4, but HR normal and mini were 2.5-fold higher than C.

In addition to these general muscle differences between the HR and C lines, other differences are specific to the subset of HR mice with the 'mini-muscle' phenotype. Depending on the muscle considered, mini-muscle individuals largely or entirely lack type IIb fibers due to a single nucleotide polymorphism (SNP) mutation in the 2b-MyHC (myh4) gene [72]. The phenotype was present at a low frequency (~7%) in the original base population, but has increased in frequency in two of the four HR lines, eventually becoming fixed in one [64,72,73]. In mini-muscle mice, the medial gastrocnemius has a reduced force per-cross sectional area [65], and the gastrocnemius has elevated capillarization [74], and hindlimb muscles have double the per-gram oxidative capacity [75]. Mini-muscle mice also have increased glycogen storage in the soleus [71]. However, mini-muscle mice do not have higher treadmill endurance, so these traits appear to not be beneficial to forced-exercise endurance.

The difference in drug response between HR and C mice suggest that pharmaceutical manipulations of serotonin receptors (5-HT_{1A} antagonism, or possibly D_4 agonism by WAY-100,635) can ablate the endurance advantage of HR mice. As the receptor we targeted is found in highest concentration in the central nervous system, we hypothesize that decreased drive to the motor neurons caused the reduction in endurance of HR mice. However, the D_4 receptor is more broadly distributed in the periphery, and D_4 is associated with human hyperactivity [76], so this interpretation is made with reservation.

The endurance values reported here are lower than those in Meek [29], even though the testing protocol in the present study was similar and started at a lower speed. This disparity is most likely attributable to the fact that Meek [29] allowed mice to "train" on wheels for ~10 days before (and during) the period of treadmill testing, whereas our mice had no access to wheels (and hence no opportunity for self-training) prior to endurance tests.

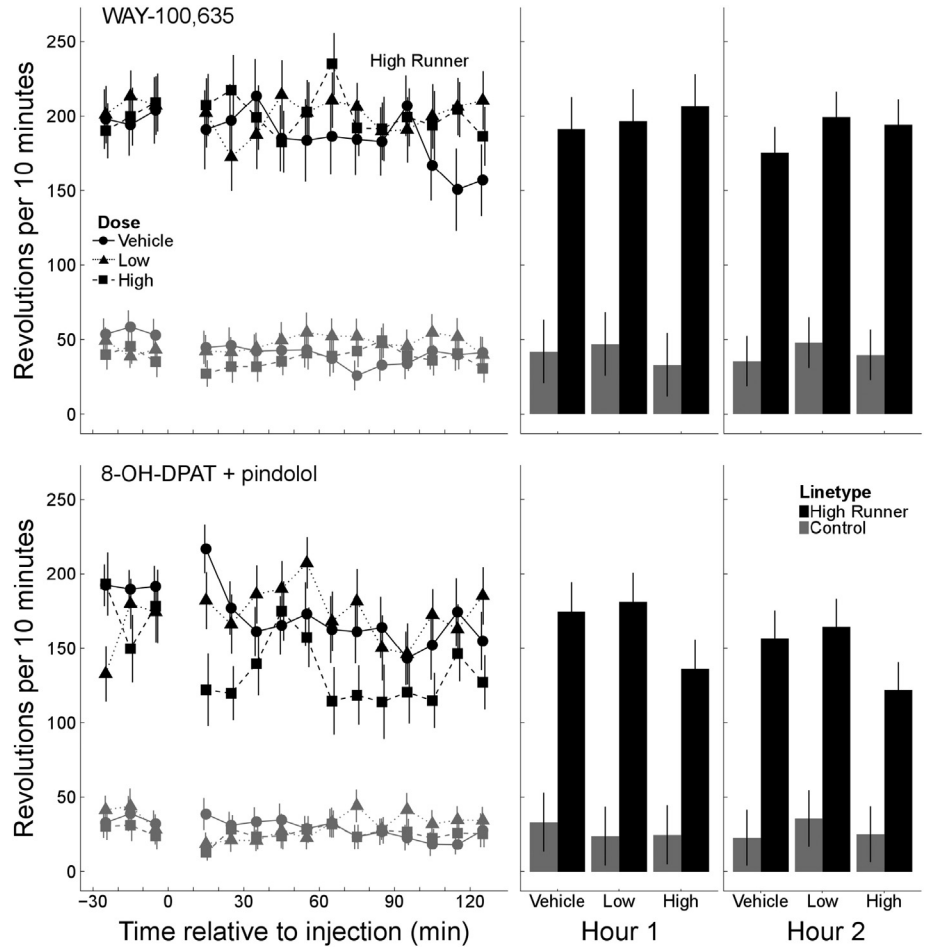

Fig. 4. Voluntary wheel running for HR and C mice before and after systemic treatment with vehicle (saline), low or high doses of either WAY-100,635 (5-HT_{1A} antagonist) (top panels) or 8-OH-DPAT + pindolol (5-HT_{1A} agonist) (bottom panels). Left panels show wheel running in 10-min bins. Time 0 corresponds with the time of injection, and the first 10 min are not included due to handling effects. Right panels group data into first and second hour following injections, as analyzed statistically (10–70 and 70–130 min, respectively). For the serotonin agonist (bottom panels), the interaction between linetype and dose was statistically significant for the first hour (see Tables 2 and 3). Values in left panels are simple means and standard errors. Values in right panels are least square means ± associated standard errors from repeated-measures (by dose) analyses in SAS Procedure Mixed, as shown in Tables 2 and 3.

4.2. Voluntary versus forced exercise

The pharmacological effects we observed differed between endurance capacity during forced treadmill exercise (measured first) and voluntary wheel running (measured after endurance trials), which suggests that the two locomotor tests are not governed by identical neurobiological processes. This possibility should be noncontroversial, because endurance trials are intended to achieve maximal physical abilities through use of external motivators [77], whereas wheel running is a "voluntary" behavior in the classical sense. Voluntary wheel running and forced treadmill exercise differ in physiological and psychological conditions. For instance, in rats, forced treadmill running induces some acute psychological stress responses, as well as chronic adaptations to stress [78], whereas wheel running generally has anxiolytic effects [79]. Two traditional biological markers of fatigue (blood glucose and lactate concentrations) suggest that the endurance protocol employed here is significantly more physically taxing than wheel running in both HR and C mice [29].

Nevertheless, given the sequential-test experimental design used here, we cannot rule out the possibility that something about the mice changed between the tests. For example, aspects of exercise physiology can change with as little as a few hours or a few days of wheel access [71,80 and references therein]. Of particular relevance for the present study, training-induced differences in 5-HT_{1A} receptor expression have been reported in rats, where 6 weeks of wheel running was sufficient to increase 5-HT_{1A} mRNA in the dorsal raphe nuclei [DRN; 32]. Although our mice had only two weeks to train on wheels before the start of injections, it seems possible that they self-trained in various ways, which in turn may have changed their drug responses. Future studies will employ a randomized testing sequence to address this possibility.

4.3. Comparison with previous findings

Our results support the findings of Ahlenius et al. [7] in that systemic WAY-100,635 injection decreased endurance in mice, and in addition we found that the effect depended on genetic background. Specifically, in our study, WAY-100,635 decreased endurance in HR mice, but not C mice. We did not replicate (to statistical significance) the finding that 8-OH-DPAT + pindolol increased endurance. In fact, our results more closely matched the biphasic effect described by Ahlenius et al. [7] when they used only 8-OH-DPAT, without the addition of pindolol, in that 8-OH-DPAT marginally (but not significantly) increased endurance in mice at the low dose, and significantly decreased endurance at the high dose. The biphasic effect of 8-OH-DPAT that we observed for endurance has been described before in the context of exhaustive exercise in rats [7] and in other behavioral contexts in multiple species [reviewed in 81]. A biphasic likely occurs because, at low doses, the agonist acts mostly presynaptically, inhibiting the release of 5-HT from the

Table 3
Voluntary wheel running over the 70–130 min post-injection. Values are least square means ± associated standard errors from repeated-measures analysis in SAS Procedure Mixed. Different sets of mice were used for the two drugs, with sample size of 49 mice per drug. A variable coding for test night (1–3) were also included in the analyses, and measure of wheel freeness was considered as a covariate, but were not a significant predictors and were ultimately not included (results not shown). P values < 0.05 are in bold font.

Variable and Drug	Dose			Dose effect	Linetype effect	Dose by linetype effect
	Vehicle	Low	High			
Total revolutions per 10 min						
WAY-100,635						
C	35.6 ± 17	48 ± 17	39.7 ± 17	F (DF) = 0.97 (2, 12)	F (DF) = 64.86 (1, 6)	F (DF) = 0.14 (2, 12)
HR	175.6 ± 17.2	199.3 ± 17.2	194.2 ± 17.2	P = 0.4055	**P = 0.0002**	P = 0.8679
8-OH-DPAT + pindolol						
C	22.9 ± 18.8	35.9 ± 18.8	25.3 ± 18.8	F (DF) = 5.08 (2, 12)	F (DF) = 24.04 (1, 6)	F (DF) = 2.3 (2, 12)
HR	156.8 ± 18.7	164.6 ± 18.7	122.2 ± 18.7	**P = 0.0252**	**P = 0.0027**	P = 0.1426
Active minutes						
WAY-100,635						
C	3.2 ± 0.8	4.3 ± 0.8	3.5 ± 0.8	F (DF) = 2.98 (2, 12)	F (DF) = 20.32 (1, 6)	F (DF) = 0.18 (2, 12)
HR	7.4 ± 0.8	8.5 ± 0.8	8.1 ± 0.8	P = 0.0892	**P = 0.0041**	P = 0.8377
8-OH-DPAT + pindolol						
C	2.3 ± 0.7	3.3 ± 0.7	2.5 ± 0.7	F (DF) = 3.49 (2, 12)	F (DF) = 25.63 (1, 6)	F (DF) = 1.02 (2, 12)
HR	7 ± 0.7	7.2 ± 0.7	6 ± 0.7	P = 0.0637	**P = 0.0023**	P = 0.3902
Average wheel speed (RPM)						
WAY-100,635						
C	4.7 ± 1.7	6.3 ± 1.7	5.5 ± 1.7	F (DF) = 1.24 (2, 12)	F (DF) = 72.71 (1, 6)	F (DF) = 0.06 (2, 12)
HR	19.4 ± 1.7	21.7 ± 1.7	21.2 ± 1.7	P = 0.3236	**P = 0.0001**	P = 0.9376
8-OH-DPAT + pindolol						
C	3.5 ± 1.9	5.0 ± 1.9	4.1 ± 1.9	F (DF) = 5.55 (2, 12)	F (DF) = 28.61 (1, 6)	F (DF) = 2.95 (2, 12)
HR	18.3 ± 1.9	19.3 ± 1.9	14.4 ± 1.9	**P = 0.0196**	**P = 0.0017**	P = 0.0907

DRN, but at high doses, it acts postsynaptically, similarly to endogenous 5-HT in other parts of the brain and spinal cord [82].

4.4. Implications for the serotonin central fatigue hypothesis

Our findings generally support the 5-HT central fatigue hypothesis (see Introduction) because pharmacological manipulation by a 5-HT_{1A} agonist and antagonist altered time to fatigue (treadmill endurance-running time) in mice. To our knowledge, this is the first time that pharmacological treatments during forced, exhaustive exercise have been compared with self-paced exercise (wheel running) for the same set of animals. As noted above, our results suggest that exhaustion in forced exercise and cessation of voluntary exercise are not governed by identical neurobiological processes.

Previous pharmacological studies of the 5-HT central fatigue hypothesis have yielded inconsistent results [see Tables 2 and 3 in 2]. The inconsistency is often attributed to differences in ambient conditions, type of exercise, type of measurement, physical fitness, other characteristics of the study individuals, or inherent differences among species [2]. Our study controlled most of those variables, with the exception of physical fitness, which is innately higher in HR mice (e.g., HR mice have higher maximal aerobic capacity), and in isolation, we found that genetically determined exercise capacity fundamentally changes how the endurance of an animal reacts to 5-HT pharmaceuticals.

Our study also provides direct evidence that the neurobiological mechanisms underlying exercise fatigue have evolved in response to selective breeding for high exercise output. Factors that are thought to contribute to central fatigue are known to respond to training [32,83], and genetically determined differences in 5-HT_{1B} receptor mRNA expression has been shown in rats which were bred for high aerobic capacity [84]. However, this is the first study to directly show an interactive effect of pharmaceuticals and genetic background on endurance ability.

Conflict of interest statement

The authors declare no conflict of interest.

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