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Opioid-mediated pain sensitivity in mice bred for high voluntary wheel running

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Abstract

We tested the hypothesis that thermal tail-flick latency, a common measure of pain sensitivity in rodents, would be altered in lines of mice that had been selectively bred for high voluntary wheel-running behavior. Specifically, we predicted that the selected (High-Runner) lines would show decreased pain sensitivity relative to their control (C; randombred) lines, and would respond differently to drugs that block opioid receptors. We first compared tail-flick latency between High-Runner and C female mice during the day (no wheel access) and at night (with wheel access). Second, we compared effects of the opioid antagonist naloxone (10 mg/kg, i.p.) on tail-flick latency during the day (no wheel access). Third, we compared effects of naloxone (5 and 10 mg/kg, i.p.) and naltrexone, a longer-lasting opioid antagonist (0.1, 1, 5, 10, 50, and 100 mg/kg, i.p.), on voluntary wheel running. Tail-flick latencies were longer at night (when mice were active on wheels), but mice from High-Runner and C lines did not differ during the day or night. Administration of naloxone (10 mg/kg, i.p.) decreased tail-flick latency measured during the day, equally in High-Runner and C mice. Naloxone (5 and 10 mg/kg, i.p.) and high doses of naltrexone (50 and 100 mg/kg, i.p.) decreased wheel running equally in High-Runner and C mice. Further studies will be required to determine whether other types of pain sensitivity have also failed to evolve in association with increased voluntary wheel running.

Keywords: Exercise; Endorphins; Naloxone; Naltrexone; Opioids; Pain sensitivity; Selective breeding; Wheel running

1. Introduction

Physical activity can induce pain and an extensive literature has described how exercise-induced pain from injuries can limit the amount or intensity of subsequent physical activity (e.g., Refs. [1,2]). In contrast, studies in both rodents and humans have demonstrated that exercise can also reduce pain [3–7]. The phenomenon in which pain perception is altered during exposure to various stressors has been referred to as stress-induced analgesia, or specifically as exercise-induced analgesia when physical activity is involved.

Over the past two decades, diminished sensitivity to pain (hypoalgesia) during and following exercise has been observed repeatedly in studies that have used a variety of noxious stimuli. These analgesic effects have been found most consistently with high-intensity aerobic exercise [2,4,8,9], but various other modes of exercise, such as resistance exercise and isometric exercise, have also been reported to be capable of modifying the sensation of pain [10,11].

Aerobic exercise is typically studied using cycling or running in humans, whereas swimming is most often used in rodents. For example, following a forced swimming protocol, responsiveness to nociceptive stimuli (e.g., heat applied to the tail or feet, electrical stimuli to the feet) decreases in both rats [12,13] and mice [14–18]. In these cases, though, whether the decreased pain perception is

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caused by the physical activity, the stressful nature of forced swimming itself, or changes in body temperature with swimming is difficult to determine [19]. In a clearer example of the effect of exercise on pain, voluntary wheel running increased the squeak thresholds of rats to pain induced by electrical stimulation of the tail, and the change in squeak threshold was positively correlated (r=0.8) with the distance run at the level of individual variation [3].

Although various mechanisms have been proposed for exercise-induced analgesia [2], most evidence suggests a role for endogenous opioids. Elevated plasma levels of endorphin and enkephalin have been found during and following exercise in humans [4,20–22], dogs [23], and rodents [24,25]. Opiate antagonists have been used to specifically examine the role of endogenous opioids in exercise-induced analgesia. In particular, naloxone and naltrexone (structural analogues of opium that compete with endogenous opioids on opiate receptors) have proven to be effective in antagonizing opioid-induced analgesia in a clinical setting [26]. If exercise-induced analgesia depends, in part, on the release of endogenous opioids, then these drugs should increase pain sensitivity during and after exercise.

Empirical studies have demonstrated that naloxone and naltrexone can reverse exercise-induced analgesia, but the effect depends on the severity and type of the exercise stress. For example, the analgesia (as indexed by the hot-plate test) induced in male Swiss–Webster mice after 3 min of forced swimming in cold water (15 °C) was not reversible by naloxone. This analgesia was considered to be non-opioid-mediated and was reversible by NMDA antagonists [27]. Other studies with forced swim tests in mice [15,18] and rats [12], though, have found that the analgesia was reversed by naloxone. This difference is probably attributable to length of exercise, water temperature, and whether the swimming was intermittent or continuous [13,17,28]. Regarding exercise in humans, the hypoalgesia produced by long-distance running was attenuated by naloxone administration [4] (see also Refs. [22,29]), whereas naloxone did not affect pain threshold after strenuous exercise as measured by finger and dental pump stimulation [30] or leg pain perception following maximal anaerobic exercise [31]. As well, forearm muscle pain following incremental handgrip exercise fatigue was not affected by naltrexone (a longer-lasting antagonist than naloxone) [32]. Of particular relevance to the present study is the report that the analgesia induced by voluntary wheel running in rats was reversible by naloxone [3]. Thus, we predicted that it would have this effect in our mice.

The purpose of the present study was to present initial studies of the relationship between exercise and one type of opioid-mediated pain sensitivity in a novel animal model—lines of house mice that have been selectively bred for high voluntary wheel running [33]. This model consists of four replicate lines that have been artificially selected for high running (High-Runner lines) as well as four lines that have been bred randomly as controls (C lines). Since generation 16, High-Runner mice have been running approximately 2.7-fold more revolutions per day as compared with C mice, mainly by running faster rather than for more minutes per day (Refs. [34,35]; see Fig. 1).

Our general working hypothesis was that pain sensitivity would be altered in the High-Runner lines. For this initial study, we specifically predicted that the High-Runner lines would show decreased opioid-mediated pain sensitivity relative to the C lines, and that they would respond differently to drugs that block opioid receptors. The first prediction is based on work in humans indicating that pain itself may limit exercise performance [2]. Hence, reduced pain sensitivity might necessarily evolve in concert with increased voluntary wheel running, and, if so, the mechanism would likely involve the opioid system. We have also found that corticosterone levels were higher in selected females in both day and night samples [36]. Although the relationship between endogenous corticosterone levels and

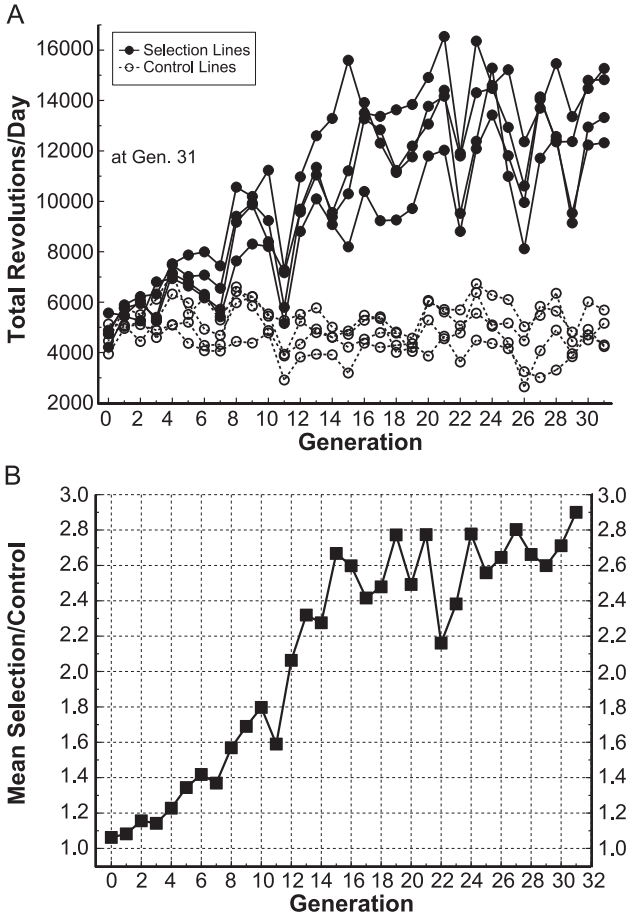

Fig. 1. (A) Cross-generation changes in average wheel running of females from eight lines of mice—four that have been selectively bred for high running (High-Runner lines) and four that have been randomly bred as controls (C lines; see Ref. [33] for original description of the experiment). Values are mean total revolutions on days 5 and 6 of a 6-day exposure to wheels. Wheel circumference is 1.12 m. (B) Mean of four High-Runner lines divided by mean of four C lines. Mice from generations 27, 29, 30, and 31 were studied here.

pain control is unclear, research suggests that corticosterone increases opioid-mediated analgesic responses [37,38]. Finally, because High-Runner mice run more and faster than C, we predicted that they might experience reduced pain sensitivity as an acute effect of their elevated activity.

We addressed these hypotheses with three experiments. In experiment I, we examined thermal tail-flick latencies of High-Runner and C mice measured during the day in animals housed without wheels and, subsequently, at night in the same animals while housed with wheels. The tail-flick test [39] has been widely used to measure pain threshold in rodents [2]. In experiment II, we administered naloxone during the day to mice housed without wheels, and tested for the effect on tail-flick latency. If the opioid system differs between High-Runner and C mice, then they might differ in the magnitude of the naloxone effect. In experiment III, we compared High-Runner and C mice with respect to the effects of naloxone and naltrexone on wheel running itself (at night). Again, differences in the opioid system should be reflected in differential responses of wheel running to opioid antagonists.

2. Materials and methods

2.1. Animals

We studied females from generations 27–31 of an ongoing artificial selection experiment for high voluntary wheel-running behavior [34,35,40]. The original progenitors were outbred, genetically variable Hsd:ICR laboratory house mice (*Mus domesticus*) purchased from Harlan Sprague Dawley in 1993 [33]. After two generations of random mating, mice were randomly paired and assigned to eight closed lines: four selected and four control. In each generation, 10 pairs of mice per line were used to propagate each of the eight lines. In all lines, offspring were weaned at 21 days of age from the dam, weighed, toe-clipped for individual identification, and housed separately in groups of four by sex until measurement of wheel running. When the offspring were 6–8 weeks old, they were housed individually with access to a running wheel for a 6-day test of voluntary running. In the selection (High-Runner) lines, the highest-running male and female from each family were chosen as breeders to propagate the lines to the next generation. The selection criterion was the total number of revolutions run on days 5 and 6 of the 6-day test. In the control (C) lines, one male and one female were chosen randomly from each family as breeders. In all lines, breeders were paired randomly, except that sibling matings were not allowed. After 16 generations of selective breeding, the level of wheel running had reached an apparent plateau of approximately 2.7-fold more revolutions per day of running in the High-Runner as compared with the C lines (see Refs. [34,35]; Fig. 1). Throughout the selection experiment and for all studies described in this paper, mice were housed routinely four per cage in a room maintained at approximately 24 °C with an alternating 12-h light–dark cycle [lights on=0800 h; lights off=2000 h, central time (CT)]. Food and water were available ad libitum throughout the experiment, and during all wheel testings.

2.2. Wheel running

As described previously [33], wheel running was measured on Whatman-type activity wheels (1.12 m circumference, 35.7 cm diameter, 10-cm-wide running surface of a 10-mm mesh bounded by clear Plexiglas and stainless steel walls; Lafayette Instruments, Lafayette, IN). Normal housing cages (27×17×12.5 cm, metal tops, wood shavings as bedding) were attached to the wheels via a 5.5-cm-long stainless steel tube inserted through a 7.7-cm-diameter hole in the side of the cage, so that a mouse in a cage had voluntary, continuous access to a wheel. A photocell counter was attached to each wheel. Customized software from San Diego Instruments (San Diego, CA) was used to collect the number of clockwise and counter-clockwise revolutions every 1-min interval for each wheel. Data were recorded continuously during wheel access, except for a 20-min period each day when data were downloaded. Wheel freeness, measured as a number of wheel rotations after spinning the wheels to a constant speed, was measured before each experiment and was included as a covariate in statistical analyses.

2.3. Pain sensitivity

We used a radiant heat tail-flick device (Tail-flick Analgesia Meter Model 33B; IITC Life Science, Woodland Hills, CA) to assess basal nociception thresholds and changes in nociceptive reactivity. A mouse was gently drawn into a wire mesh tube, with the tail hanging out freely from the tube and positioned immediately above the photocell for radiant heat stimulation on the dorsal surface of the middle of the tail. Intensity of the heat source was set to produce baseline tail-flick latencies between 3 and 6 s, measured to the nearest 0.01 s. To avoid tissue damage, a trial was terminated if a response did not occur within 10 s [41,42]. For each mouse, tail-flick latency was measured five times consecutively, with 10-s intervals between measurements. The mean of the longest four values was then analyzed for each individual.

2.4. Drug administration

Injection solutions of naloxone hydrochloride and naltrexone hydrochloride (Sigma, St. Louis, MO) were freshly prepared each day by dissolving the anhydrous drug in 0.9% saline solution. Naloxone was administered intraperitoneally at doses of 10 mg/kg (experiment II), and 5 and 10 mg/kg (experiment III). Naltrexone was administered intraperitoneally at doses of 0.1, 1, 5, 10, 50, and 100 mg/kg

(experiment III). In all experiments, injection volume was adjusted to the body mass of the animal (0.005 ml/g).

2.5. Experimental procedures

Experiment I involved measurement of baseline tail-flick latency during the day (without wheels) and at night (with wheels). Approximately nine mice from each line (one per family, n=72 total) were randomly chosen from generation 29. Daytime baseline tail-flick latency was measured twice, on two consecutive days, when mice were approximately 42 days of age. Measurements began at 1500 CT, when most of the mice are inactive. Testing order was randomized, and the same sequence was used on the second day. One week after these measurements, mice were given access to wheels for 6 days as part of the regular selection protocol (see Ref. [33]). The subjects for this study were then allowed to remain with wheel access for an additional day. During the seventh night, tail-flick latency was measured once during peak wheel-running hours (i.e., 2–3 h after lights off; e.g., see Refs. [36,40]).

Experiment II determined the effect of naloxone on tail-flick latency during the day in mice housed without wheels. Nonbreeder females from generation 31 were used. Because exclusion of the top runners (which were used as breeders) would have caused the sample from the selected lines to be biased downwards with respect to wheel running, we also excluded the lowest-running animals in selected-line families. Of the remaining mice, one female from each of six families per line was randomly chosen (n=48 total). At the time of testing, mice were approximately 18 weeks of age and had undergone the routine 6-day test of wheel exposure (see Ref. [33]) approximately 11 weeks prior to testing. During the day, each mouse received both naloxone (10 mg/kg) and saline in a randomly determined order, with the injections separated by 48 h. The saline injection has been reported as a valid control in comparing thermal stimulus response to naloxone in deer mice [43,44]. Because of time constraints, only half of the study group was treated on a given day, such that the study was conducted over 4 days. Tail-flick latency was measured 30 min following administration of drug or saline, and the difference in values was analyzed.

Experiment III comprised several measurements of the effects of naloxone and naltrexone on wheel running (at night). Mice from three different generations were sampled. For naloxone trials, breeders of generation 26 were allowed to produce a second litter, 5 weeks after they weaned their first litters, which was part of the routine selection protocol. Females from these second litters (generation 27B) were used. Six females were randomly chosen from each line (n=48 total). They were given access to running wheels at approximately 56 days of age, and maintained with wheel access for 7 weeks. Testing began on day 50 of wheel access. Each individual received all three treatments (saline, 5 mg/kg, and 10 mg/kg naloxone) in a randomly determined order over the course of 6 days, with 48 h between each injection to avoid carryover effects of the previous treatment. The doses of 5 and 10 mg/kg naloxone have been previously reported to maintain a physiologically effective blockade of opiate receptors in rats [45]. A mouse received treatment at approximately the same time of day for each injection. Injections began 2 h after lights off, which is during peak wheel-running activity [36,40]. The acute locomotor response was measured as the total number of wheel revolutions in the period from 10 to 50 min postinjection [45]. The first 10 min were excluded because saline injection decreases wheel running during this period [34].

Mice from generation 30B were used for the low-dose and medium-dose naltrexone trials. Six females were randomly chosen from each line (n=48 total), then placed in cages with access to running wheels when they were approximately 4 weeks old. After 4 weeks of acclimation, mice were injected with vehicle (0.9% saline), 0.1 mg/kg naltrexone, or 1 mg/kg naltrexone. Injection sequences were as described above for naloxone, except that injections began at 1 h and 20 min before lights off (rather than 2 h after lights off), so that all the injections were completed by the time lights went off. The acute locomotor response to treatment was measured as the total number of wheel revolutions in the 100-min period from 100 to 200 min postinjection (a period that overlaps with that analyzed for naloxone). A longer interval was chosen for naltrexone (100 min) than naloxone (40 min) because naltrexone is a long-acting opioid antagonist (effects last more than 3 h in rats) [46]. Three days later, the same mice were used for medium-dose naltrexone trails. Mice were injected with vehicle (0.9% saline), 5 mg/kg naltrexone, or 10 mg/kg naltrexone. Injection sequences were as described for the low-dose trials, and the same interval (100–200 min postinjection) was used to measure acute locomotor responses.

For the high-dose naltrexone trials, we used the same nonbreeder female mice from generation 31 as were used in experiment II. At the time of testing, they were approximately 24 weeks of age and had been maintained with wheel access for 2 weeks. Each individual received all three treatments (saline, 50 mg/kg, and 100 mg/kg naltrexone) in a randomly determined order, as described above. The acute locomotor response to treatment was measured as the total number of wheel revolutions in the 120-min period from 10 to 130 min postinjection. The period started at 10 min postinjection rather than 100 min postinjection (used for the lower doses) because the high doses of naltrexone were administered 2 h after lights off (rather than 1 h and 20 min before lights off) and we wished to measure effects of the drugs during peak activity, after lights off. A 120-min period was used rather than a 100-min period because inspection of the data indicated that the higher doses of naltrexone affected wheel running over a period longer than 100 min.

2.6. Statistical analyses

Statistical analyses were performed using SPSS for Windows (General Linear Model—Univariate Function), version 10.0. The general statistical model for comparison of High-Runner with C lines was always a mixed model with line nested within linetype (High-Runner vs. C) considered as a random effect (SPSS employs least squares, not restricted maximum likelihood), and degrees of freedom for testing the effect of linetypes always being 1 and 6.

For experiment I, we also used a paired *t* test to compare day and night tail-flick latency, and we computed Pearson's *r* to index the consistency of individual differences between day and night. For experiment II, similar analyses were performed, but for values following saline or naloxone injection, rather than day versus night. For experiment III, which tested whether naloxone or naltrexone had a differential effect on wheel running in High-Runner versus C mice, we first analyzed absolute wheel-running responses to naloxone or naltrexone injections separately for High-Runner and C lines to determine the effect of the drug doses within each linetype. One-way ANOVAs were used, with individual mice entered as a blocking factor (because the doses were applied to the same individual). Wheel freeness and body mass initially were considered as covariates, but were excluded from final analyses because they were never statistically significant. We also analyzed proportional responses (response after drug divided by response after saline, following Rhodes et al. [34] separately for each drug dose). To improve normality of residuals, the proportional responses were transformed by raising to an exponent <1 (residuals were highly positively skewed otherwise).

3. Results

Fig. 1 shows the difference in wheel running between High-Runner and C lines (females) from the beginning of the selective breeding through generation 31. Note that, as measured on days 5 and 6 of a 6-day exposure to wheels, the differential has remained approximately constant since generation 16. For the generational span over which the present experiments were conducted (generations 27–31), the grand mean wheel running was 13,072 rev/day for females from the selected lines versus 4771 for the control lines, which is a factorial difference of 2.74.

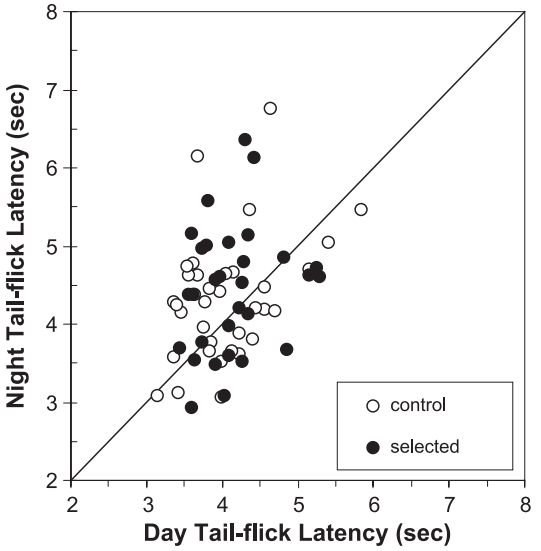

Fig. 2. Individual values of baseline tail-flick latency during the day (without wheels) plotted against values at night (with wheels). Line of identity is shown. Night values are significantly longer, on average, and the correlation between day and night values is statistically significant for mice from C lines and for all mice pooled, but not for mice from the High-Runner lines alone (Table 1).

3.1. Experiment I

Tail-flick latency (Table 1) did not differ between High-Runner and C lines during the day ($F_{1,6}$=0.339, P=0.581) or at night ($F_{1,6}$=0.190, P=0.678). Night values were longer than day values (Table 1, Fig. 2) for both High-Runner (P=0.046) and C (P=0.058) mice (P=0.006 for all mice pooled). Day and night measurements were significantly correlated within subjects for all mice (P=0.011) and for C mice alone (P=0.040), but not for High-Runner mice alone (P=0.209).

Table 1
Experiment I: comparison of tail-flick latency in mice selected for high voluntary wheel running and in randombred control lines

Linetype	Mean±S.E. (s)		Day vs. night				
	Day[a]	Night[b]	Difference			Correlation	
			Paired *t*	*df*	Two-tailed *P*	*r*	Two-tailed *P*
High-Runner	4.22±0.096 (*n*=33)	4.47±0.128 (*n*=31)	2.08	30	0.046	0.232	0.209
Control	4.06±0.109 (*n*=35)	4.31±0.151 (*n*=34)	1.96	33	0.058	0.353	0.040
Pooled	4.14±0.073 (*n*=68)	4.49±0.100 (*n*=65)	2.87	64	0.006	0.314	0.011

Effects of linetype on TFL during the day or night were analyzed using a mixed-effects nested model in SPSS, with linetype entered as a fixed factor and line nested within linetype as a random factor.
Body mass was considered as a possible covariate; however, it was never significant and therefore the *P* values shown in this table refer to analyses without body mass as covariate.
[a] Effects of selection on TFL during the night: $F_{1,6}$=0.19, P=0.678.
[b] Effects of selection on TFL during the day: $F_{1,6}$=0.34, P=0.581.

Table 2
Experiment II: effects of naloxone (10 mg/kg) on tail-flick latency during the day

Linetype	*n*	Mean±S.E. (s)		Saline vs. naloxone				
		Saline	Naloxone	Difference		Two-tailed *P*	Correlation	
				Paired *t*	*df*		*r*	Two-tailed *P*
High-Runner	22	5.20±0.127	4.49±0.111	5.49	21	<0.001	0.417	0.054
Control	23	5.30±0.190	4.52±0.181	8.09	22	<0.001	0.866	<0.001
Pooled	45	5.25±0.114	4.50±0.106	9.40	44	<0.001	0.743	<0.001

Effects of linetype on TFL were analyzed using a mixed-effects nested model in SPSS, with linetype entered as a fixed factor and line nested within linetype as a random factor.
Body mass was considered as a possible covariate; however, it was never significant and therefore the *P* values shown in this table refer to analyses without body mass as covariate.
Effects of selection on TFL response difference (saline–naloxone value): $F_{1,6}$=0.34, *P*=0.583.

For the night values, we also performed nested analysis of covariance with amount of wheel running as a covariate. We used total revolutions in the periods 20, 40, 60, 80, 100, and 120 min prior to measurement. In all cases, the difference between High-Runner and C mice remained nonsignificant. Wheel revolutions tended to have a negative correlation with tail-flick latency, and *P* values were 0.117, 0.076, 0.071, 0.059, 0.056, and 0.042, respectively. Thus, at the level of individual variation within lines, higher amounts of running tend to be weakly associated with shorter tail-flick latencies (increased pain sensitivity). Note that this is contrary to the prediction that the acute effect of exercise decreases pain sensitivity [3–7].

3.2. Experiment II

After administration of naloxone (10 mg/kg, i.p.), both High-Runner and C mice exhibited shorter tail-flick latencies compared to the saline injection (both *P*<0.001), and the difference in latency (saline–naloxone value) did not differ between High-Runner and C lines (*P*=0.583) (Table 2, Fig. 3). At the level of individual variation, postsaline and postnaloxone values were positively correlated (Table 2, Fig. 3).

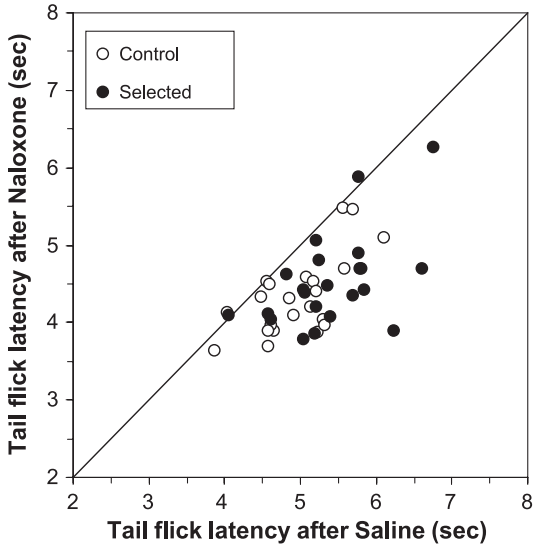

Fig. 3. Individual values of tail-flick latency during the day (no wheels) following saline injection of naloxone (10 mg/kg, i.p.). Values for individual mice are significantly correlated, and both selected and control mice show significant reductions in latency following naloxone. However, the response (saline–naloxone value) does not differ between the two linetypes (Table 2).

3.3. Experiment III

As shown in Table 3 and Fig. 4, naloxone (5 or 10 mg/kg) decreased wheel running in both control and High-Runner mice as indicated by results of the two-way ANOVA (*P*=0.006 for dose, *P*<0.001 for linetype, *P*=0.124 for interaction). However, when considering High-Runner lines alone, the *P* value (0.120) did not reach statistical significance as it did in control mice (0.028). Note that mice from the High-Runner lines always ran more than controls, irrespective of dose. The magnitude of the naloxone response, measured as a proportion of baseline running (after the saline injection), did not differ significantly between High-Runner and C mice for either dose (Table 4).

Low and medium doses of naltrexone (0.1, 1, 5, and 10 mg/kg) had no measurable effect on wheel running in either C or High-Runner lines (Table 3). High doses (50 or 100 mg/kg) decreased wheel running in a dose-dependent manner in both C and High-Runner mice (Table 3, Fig. 4), but the magnitude of the decreases (measured as a proportion of baseline running after the saline injection) did not differ significantly between C and High-Runner lines (Table 4). Mice from High-Runner lines always ran more than controls, irrespective of dose (Table 3).

4. Discussion

Females from four replicate lines of mice artificially selected for high voluntary wheel-running behavior (High-Runner lines) did not show decreased pain sensitivity, as measured by tail-flick latencies, or altered responsiveness to opioid antagonists relative to their unselected control (C) lines. Thus, opioid receptors appear to function similarly in

Table 3
Experiment III: effects of naloxone and naltrexone on voluntary wheel-running behavior (absolute responses)

Drug	Dose	One-way ANOVA for drug effects in each linetype		Two-way ANOVA for drug and selection response	
		Control	High-Runner	Sources	All lines together
Naloxone	Saline	376±42.7	1012±85.2	Dose	($F_{2,72}$=5.41, P=0.006)
	5 mg/kg	277±37.1	905±74.7	Linetype	($F_{1,6}$=45.95, P<0.001)
	10 mg/kg	295±48.8	888±97.7	Dose×Linetype	($F_{2,72}$=2.15, P=0.124)
		($F_{2,38}$=3.94, P=0.028)	($F_{2,38}$=2.25, P=0.120)		
Naltrexone (low dose)	Saline	1072±100.6	3962±320.7	Dose	($F_{2,78}$=0.31, P=0.732)
	0.1 mg/kg	1024±106.4	3906±304.6	Linetype	($F_{1,6}$=40.85, P<0.001)
	1 mg/kg	1072±94.9	3793±260.3	Dose×Linetype	($F_{2,78}$=0.08, P=0.920)
		($F_{2,40}$=0.20, P=0.817)	($F_{2,38}$=0.57, P=0.569)		
Naltrexone (medium dose)	Saline	916±114.9	3479±296.5	Dose	($F_{2,68}$=0.51, P=0.600)
	5 mg/kg	904±94.6	3504±326.9	Linetype	($F_{1,6}$=14.69, P=0.008)
	10 mg/kg	872±87.5	3468±316.6	Dose×Linetype	($F_{2,68}$=0.69, P=0.506)
		($F_{2,34}$=0.02, P=0.980)	($F_{2,34}$=0.26, P=0.770)		
Naltrexone (high dose)	Saline	1172±119.9	2952±236.6	Dose	($F_{2,66}$=30.75, P < 0.001)
	50 mg/kg	943±153.2	2653±267.3	Linetype	($F_{1,6}$=18.89, P=0.005)
	100 mg/kg	555±96.4	1627±243.7	Dose×Linetype	($F_{2,66}$=0.51, P=0.603)
		($F_{2,34}$=11.16, P<0.001)	($F_{2,32}$=27.63, P<0.001)		

Values are mean±S.E. for total revolutions 10–50, 100–200, and 10–130 min postinjection for naloxone, naltrexone (low dose and medium dose), and naltrexone (high dose), respectively.
Date were analyzed two ways: (1) separately for C and High-Runner mice with dose as a repeated-measures factor and line as a random effect; (2) by two-way nested ANOVA with dose and linetype as fixed factors, and line as a random effect.
Body mass and wheel freeness were considered as a possible covariates, but were never significant; therefore, P values refer to analyses without covariates.

High-Runner and C mice with regard to pain sensitivity and wheel running. Other nonopioid-mediated forms of pain sensitivity might still have changed in the High-Runner mice, but that will require future investigation. Nonetheless, our current results are important because they suggest that the opioid system probably does not play a key role in determining levels of voluntary physical activity in our model. In contrast, other systems that involve the neuromodulator dopamine [34,47] and the steroid hormone corticosterone [36] do appear to have changed in the High-Runner lines. Thus, the present negative results are an important piece of an emerging picture of the phenotypic changes that have taken place in association with increased physical activity in the replicate lines of mice.

The decreased pain sensitivity (increased tail-flick latency) at night relative to that during the day in both

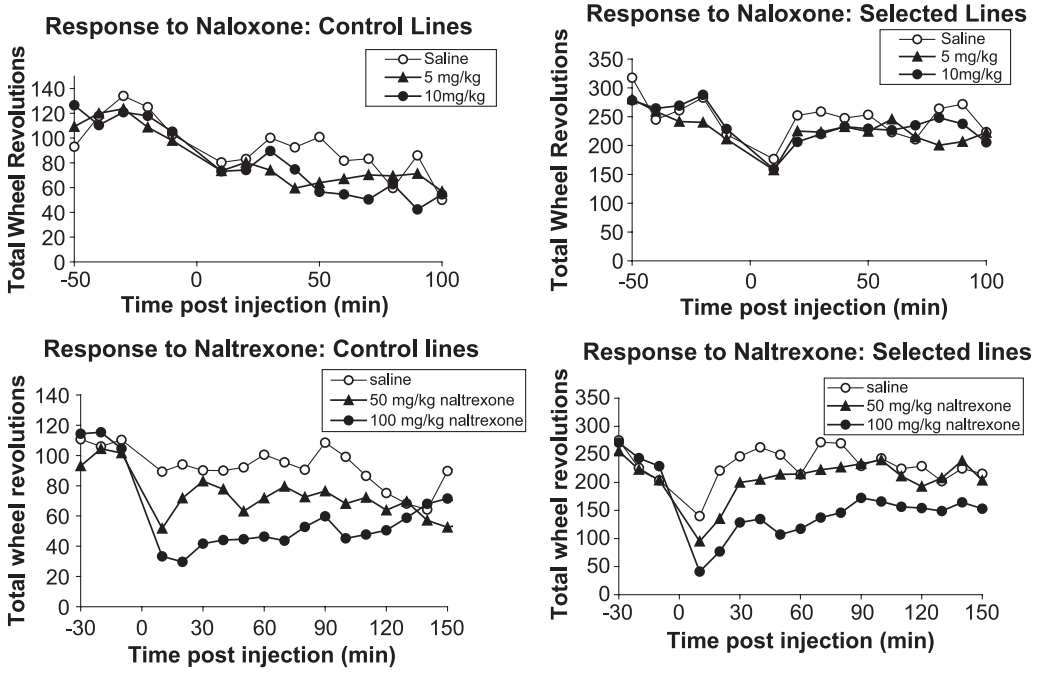

Fig. 4. Wheel-running response to intraperitoneal administration of naloxone (10–50 min postinjection) and high doses of naltrexone (10–130 min postinjection). Both opioid antagonists decreased wheel running in both C and High-Runner lines (Table 3), but the proportional response did not differ significantly between High-Runner and C lines (Table 4).

Table 4
Experiment III: effects of naloxone and naltrexone on voluntary wheel-running behavior (proportional responses)

Drug	Dose	Control	High-Runner	Transform	$F_{1,6}$	P
Naloxone	5 mg/kg	0.74 (0.50, 1.06)	0.89 (0.79, 1.00)	^0.2	1.86	0.221
	10 mg/kg	0.71 (0.43, 1.06)	0.84 (0.70, 0.99)	^0.5	0.93	0.373
Naltrexone (low dose)	0.1 mg/kg	1.00 (0.87, 1.12)	0.99 (0.92, 1.06)	None	0.01	0.932
	1 mg/kg	0.97 (0.76, 1.23)	0.98 (0.87, 1.09)	^0.2	0.03	0.880
Naltrexone (medium dose)	5 mg/kg	1.04 (0.92, 1.17)	0.97 (0.89, 1.06)	^0.01	0.84	0.394
	10 mg/kg	1.02 (0.74, 1.37)	0.91 (0.79, 1.04)	^0.2	0.36	0.570
Naltrexone (high dose)	50 mg/kg	0.86 (0.65, 1.08)	0.91 (0.78, 1.03)	None	0.48	0.513
	100 mg/kg	0.47 (0.34, 0.59)	0.52 (0.41, 0.64)	None	0.64	0.452

Values are backtransformed means and 95% confidence intervals for proportional responses (computed as wheel revolutions following drug injection divided by revolutions following saline injection).

High-Runner and C mice (Table 1, Fig. 2) is consistent with exercise-induced analgesia [3,9] because the mice were exercising on running wheels at night but not during the day (mice from both High-Runner and C lines run on wheels mainly at night, as shown in Fig. 1 of Refs. [36,40,47]). However, this comparison confounds a diurnal rhythm in pain sensitivity, which has been reported in house mice not housed with running wheels (e.g., Refs. [48,49]), with any effects of voluntary exercise per se. In our study, we can attempt to tease apart these factors by examining the correlation between level of running and pain sensitivity among individuals *within the High-Runner and C lines* that ran varying distances at night. We looked at a variety of different time scales (total distance run 20, 40, 60, 80, 100, and 120 min prior to measurement) and, in all cases, the analyses showed evidence of weak *negative* (rather than positive) correlations between level of wheel running and tail-flick latency, such that animals that were relatively more active tended to be *more*, not less, sensitive to pain. Taken together, these results suggest that the decreased pain sensitivity at night might indeed be related to the diurnal rhythm rather than effects of exercise per se.

Naloxone decreased tail-flick latency similarly in High-Runner and C mice (Table 2, Fig. 3), which suggests that the function of opioid receptors in pain sensitivity has not evolved in the selected lines. The increased pain sensitivity in response to naloxone is consistent with a role for endogenous opioids in pain sensitivity [48,50,51]. Typically, naloxone is used to reverse effects of morphine [52–54], and there is disagreement about whether naloxone, by itself, can increase pain sensitivity in rodents. For example, two studies in rats [55,56] and one study in laboratory mice [43] found no effect of naloxone on baseline tail-flick latency, but another study in rats [52] and one in laboratory mice [48] found that naloxone increased baseline pain sensitivity. Our results clearly demonstrate that naloxone increases baseline pain sensitivity in these lines of house mice (as measured by tail-flick latency) relative to a saline injection.

High-Runner and C mice were not only similarly sensitive to effects of naloxone on tail-flick latencies, but they also showed a similar sensitivity to the effects of naloxone and naltrexone on wheel-running behavior. Naloxone and naltrexone reduced wheel running in a dose-dependent manner, to a similar extent (measured as a proportion of baseline running) in High-Runner and C mice, thus providing further evidence that the function of endogenous opioids in wheel running has not evolved in the selected lines. One function of endogenous opioids in wheel running could be to reduce pain associated with wheel running. Therefore, the result that opioid antagonists affect wheel running equally in High-Runner and C mice is consistent with the observation that pain sensitivity does not differ between High-Runner and C mice. Alternatively, endogenous opioids may be the reward perceived from wheel running, which motivates animals to run [57]. Neural systems that motivate running are among the most likely candidates to have evolved in the High-Runner mice because the selective breeding was conducted on a voluntary behavior [34,47,58]. However, recent evidence distinguishes the reward (or the liking of a stimulus) from the motivation to receive the reward (or the wanting of a stimulus) [59,60]. The reward may involve endogenous opiates, while substrates for motivation may involve dopamine. Therefore, it is possible that the rewarding aspects of wheel running, involving opioid function, have not evolved in High-Runner mice, whereas the motivation to receive the reward, involving dopamine function, has evolved [34,47,58].

The decrease in wheel running in High-Runner and C mice after administration of naloxone or naltrexone (Tables 3 and 4, Fig. 4) is consistent with results for rats (naloxone [61,62]) and hamsters (naltrexone [63]), but we know of no previous studies of mice. The decrease in running could be mediated by a variety of mechanisms. It is possible that the opioid antagonists blocked the reward [62,64–66] from wheel running, thus causing reduced motivation for running. However, as discussed above, it is unlikely that the opioid antagonists affected motivation to run because in this case, High-Runner mice should have responded differently than controls because of differences in motivation. This is consistent with Belke and Dunlop [67], who suggest that the high doses of naltrexone needed to reduce wheel running in rats are unlikely to affect motivation for running but rather reduce wheel running via malaise, motor impairment, or sedation effect. Although we did not measure

endorphin levels, the running distances in our experiment were similar to those exhibited by rats in which elevated β-endorphin levels were found [68]; comparable data are not available for mice. Therefore, it is possible that the opioid antagonists reversed a reduction in pain perception during exercise [3,15,18], which resulted in reduced wheel running because running became painful. Furthermore, the opioid antagonists might have reduced wheel running by blocking endorphin-mediated glucose uptake [69], or by blocking endorphin-mediated delay of fatigue [70].

In conclusion, our experiments suggest that decreased pain sensitivity (as measured by tail-flick latency) has not evolved in lines of mice that have been selectively bred for high voluntary wheel running. Moreover, we find no evidence for a change in opioid function with regard to either pain sensitivity or wheel running. However, we caution that the present studies involved only females and may or may not apply to males (e.g., see Ref. [71] and references therein). Nonetheless, it is possible that the changes in neuronal function that cause the increased running may be restricted to systems that control motivation (such as dopamine [34,47,58]), which may be separate from those involved with natural reward (such as opioids [59,60]).

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