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Mice from lines selectively bred for high voluntary wheel running exhibit lower blood pressure during withdrawal from wheel access

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HIGHLIGHTS

- Exercise is rewarding and has positive effects on mental and physical health.
- Excessive exercise may sometimes reflect an underlying addiction.
- We studied lines of mice selectively bred for high voluntary wheel running (HR).
- During withdrawal from wheel access, HR had lower blood pressure than controls.
- Mice from the selectively bred HR lines may be addicted to wheel running.

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ABSTRACT

Exercise is known to be rewarding and have positive effects on mental and physical health. Excessive exercise, however, can be the result of an underlying behavioral/physiological addiction. Both humans who exercise regularly and rodent models of exercise addiction sometimes display behavioral withdrawal symptoms, including depression and anxiety, when exercise is denied. However, few studies have examined the physiological state that occurs during this withdrawal period. Alterations in blood pressure (BP) are common physiological indicators of withdrawal in a variety of addictions. In this study, we examined exercise withdrawal in four replicate lines of mice selectively bred for high voluntary wheel running (HR lines). Mice from the HR lines run almost 3-fold greater distances on wheels than those from non-selected control lines, and have altered brain activity as well as increased behavioral despair when wheel access is removed. We tested the hypothesis that male HR mice have an altered cardiovascular response (heart rate, systolic, diastolic, and mean arterial pressure [MAP]) during exercise withdrawal. Measurements using an occlusion tail-cuff system were taken during 8 days of baseline, 6 days of wheel access, and 2 days of withdrawal (wheel access blocked). During withdrawal, HR mice had significantly lower systolic BP, diastolic BP, and MAP than controls, potentially indicating a differential dependence on voluntary wheel running in HR mice. This is the first characterization of a cardiovascular withdrawal response in an animal model of high voluntary exercise.

1. Introduction

Physical exercise can be a self-rewarding behavior, and it has been hypothesized to have addictive properties [1–3]. Exercise addiction has received limited attention in humans [3–6], and even then, the primary focus has been on the psychosocial ramifications [3,4,7]. Studies examining exercise addiction in non-human animals have done so primarily in the context of its biomedical significance as a condition plaguing a subset of human anorexia patients (i.e., "activity anorexia", reviewed in [8]).

Evidence suggests that exercise addiction involves the brain's reward pathway, as do other forms of addiction [6,9–13]. In humans, for example, bouts of moderate exercise can help attenuate symptoms of both nicotine and alcohol withdrawal [9–11,14,68]. This observation implies that exercise can be highly motivated (although obviously with variation among individuals and among species), and that the pursuit of or engagement in exercise can substitute for aspects of chemical addiction. Moreover, after withdrawal from exercise, some studies have found signs of depression and anxiety, or other indications of negative affective states in both mice and humans [6,15,16].

Two common physiological indicators of withdrawal are alterations in blood pressure (BP) and heart rate, with the former previously shown to respond to artificial selection in laboratory house mice [17]. Alterations in these two indicators at rest have been observed over an

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array of withdrawal disorders in humans [6,18–20], rats [21–23], and mice [24]. Withdrawal symptoms vary with the type of addiction. Studies of nicotine withdrawal in humans report decreased heart rate and systolic blood pressure (SBP) [18,25–27]. Conversely, alcohol withdrawal causes an increase in both SBP and heart rate in humans [19,28–30]. Rats undergoing opiate withdrawal [15,22,31,32] display elevated BP but highly variable heart rate. Although each of these withdrawal syndromes is unique, all result in altered resting SBP. Additionally, one human study [6] measured resting heart rate during withdrawal from high levels of exercise, and found it to be elevated. To our knowledge, no study has evaluated BP during withdrawal from exercise. Furthermore, given that BP has a genetic component and that lines of mice selectively bred for BP extremes exhibit a number of correlated responses (e.g., [33–36]), we might expect that selection for a behavior known to alter cardiopulmonary function may result in concomitant changes in BP.

We studied lines of mice bred for high voluntary wheel running, which is a self-rewarding behavior in rodents (reviewed in [37,38]). At the time of the present study, these mice had been selectively bred for over 40 generations from an original base population of outbred Hsd:ICR mice [39–42]. As a result of the selective breeding, the four replicate high-runner (HR) lines of mice run nearly 3-fold farther per day as compared with the four non-selected Control (C) lines, at higher speeds [41] and in a more intermittent fashion [43]. Pharmacological evidence suggests that alterations in dopamine [44,45] and endocannabinoid ([46], and [78]) signaling underlie the elevated wheel running of HR mice. When housed without wheels, HR mice exhibit elevated home-cage activity [16]. During exercise withdrawal (removal of wheels following several days of access), HR mice exhibit altered brain activity ([47]; only females were studied) and elevated behavioral despair ([16]; males only).

HR mice have larger heart ventricular mass than C mice [48,49], which could have implications for stroke volume, cardiac output, and BP. Likewise, elevated blood hemoglobin concentrations following injection of an erythropoietin (EPO) analog are associated with a larger spleen mass in both line types [49]. The spleen is an erythrocyte storage and processing center that contributes to blood viscosity, and can play an indirect role in BP. Therefore, heart ventricular mass and spleen mass were also measured in the present study.

Additionally, a Mendelian recessive allele that results in a 50% reduction in hindlimb muscle mass and a doubling of mass-specific aerobic capacity currently exists in two of the HR lines [50–52]. This "mighty mini-muscle" phenotype also results in a smaller body mass, a larger heart ventricle mass (when corrected for body mass), and higher muscle capillarity ([53,54], references therein). Previously, we hypothesized that mini-muscle individuals might experience higher BPs that had led to cardiac hypertrophy [52]. Therefore, in the present study, mini-muscle status was used as an additional factor in the statistical analysis of BP.

2. Materials and methods

2.1. Selection experiment and housing

House mice (*Mus domesticus*) from generation 41 of the selection experiment for high voluntary wheel running were used in this study [39,40]. A total of 72 males (44 HR; 28 C) were weaned at 3 weeks of age and housed 4 per cage. One of the HR lines (lab designation #6) remains polymorphic for the mighty mini-muscle trait [50–52], so we included a larger sample size (N = 23) from that line. All other HR and C lines had equal representation (N = 7/line). Four animals died during the course of the study, and the final sample size at the time of dissection was N = 68. The causes of these deaths were not conclusively identified, although some appeared to be related to failures with water bottles. In any case, the mortality occurred outside the range of testing ages, and so far as we could ascertain was not related to any of the testing procedures. Mice were re-housed individually two days before the start of the baseline measurements, and remained so throughout the course of the experiment. A 12-hour light/dark cycle (lights on: 0700, lights off: 1900) was maintained at all times, and mice were given food (Harlan Teklad Rodent Diet 8604 [W], Harlan Teklad, Madison, WI, USA) and water ad libitum. Daily room temperature was measured and the average room temperature was 22 °C (range: 21–23 °C).

2.2. Experimental design

Mice were separated into three batches (N = 24/batch) to keep BP measurements within a standardized 4-hour daily time range and minimize any time-of-day effects. Each mouse underwent 16 days of BP testing in three sequential phases (measurements were obtained every day on all mice, regardless of treatment group). First, mice were given 8 days of baseline BP measurements (days 1–8) without wheel access ('baseline' phase). Next, mice were given wheel access for 6 days (days 9–14; 'wheel access' phase). Finally, for the last two days ('withdrawal' phase), half of the mice (selected randomly) had their wheel access blocked and half were allowed continued wheel access (days 15–16; Fig. 1). BP measurements during the baseline, wheel access, and withdrawal phases were made on batch 1 before proceeding to batch 2 and batch 3. An effort was made to assign younger mice to later batches to offset any potential age differences due to the 8-week interval between the start of batch 1 and the start of batch 3. Nevertheless, age was used as a covariate in all final statistical analyses.

In this study we chose to evaluate cardiovascular characteristics after a 6-day exposure to wheels. We acknowledge that mice may take longer periods (up to 2–3 weeks) to acclimate to running wheels and reach a plateau with regard to running distance. A number of factors may contribute to the acclimation process causing day-to-day variation among individuals in the trajectory of initial wheel-running behavior. We chose the current paradigm to reflect as accurately as possible the conditions under which the HR mice were selectively bred. Although initial "learning curves" may vary among strains of mice, this does not seem to be the case for HR mice compared with ICR control mice (see Fig. 5 in [55]).

All BP measurements were made between 1000 and 1500 h daily, with six mice tested simultaneously. The entire procedure lasted approximately 1 h for each group of six mice. Therefore, each batch of 24 mice completed the BP measurements within a 4-hour period each day. The sequence of testing and the cohort to which a given mouse belonged were randomly selected each day. Preliminary analyses revealed that cohort (i.e., time of day) was never a statistically significant predictor of any measure.

After the final day of BP measurements, mice were euthanized and body mass, body length, heart ventricle mass, spleen mass, and presence/absence of the mini-muscle phenotype were ascertained. Mini-muscle status was determined via dissection and mass of the triceps surae muscle group.

2.3. Blood pressure measurements

BP was non-invasively measured by determining the tail blood volume with a volume pressure-recording sensor and an occlusion tail-cuff (CODA 6 System, Kent Scientific, Torrington, CT). Mice were warmed with heating lamps for 15 min prior to the start of the BP trial to increase peripheral circulation [56–62]. Mice were then placed into restraint tubes and BP tail cuffs were attached. In the literature on BP in mice, the number of measurement cycles per day varies from 3 to 30 [57–62]. We chose 30 cycles to maximize the number of measurements we could obtain within the given time constraints. The first 10 cycles were considered to be the acclimation and were not used in the final analysis. The remaining 20 were evaluated and excluded if they did not meet specific inclusion criteria based on tail

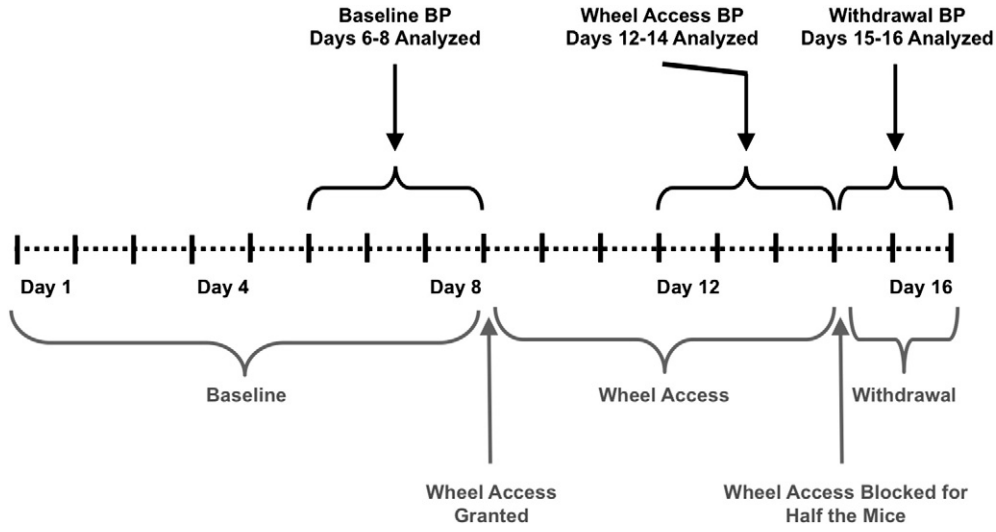

Fig. 1. Experimental design timeline, including days of baseline BP measurements, wheel access, and withdrawal phases, as well as the specific days within each time period for which the data analysis was performed. BP measurements were taken on each of the 16 days. In addition to BP measures, wheel running was measured every day but was analyzed and reported for the last two days of the wheel-access phase only.

blood flow, heart rate, and integrity of the inflation cuff pressure traces. The inclusion criteria were as follows: tail blood flow above 20 μl/min, heart rate below 900 beats/min, and absence of "noise" due to tail movement or improper tail cuff inflation. These criteria were derived from the BP literature [56–62] as well as manufacturer recommendations (CODA System, Kent Scientific, Torrington, CT). In addition, statistical outliers were removed as indicated below in the Statistical analyses section.

Sixty-second intervals separated each of the 30 measurement cycles, with a cumulative measurement time of approximately 45 min/mouse/day. Following each trial, mice were weighed and body temperature readings were taken via a digital rectal thermometer (±0.1 °C). Both body mass and body temperature were used as covariates in the final analyses.

BP measurements were taken for every mouse on all 16 days of the study. During the 8 days of baseline, only the measurements from the final three days (days 6–8) were used to assess baseline BP. The first 5 days served to estimate repeatability of the measurements (results not shown). During the 6-day wheel access phase, only the final three days (days 12–14) were used to calculate BP. During the withdrawal phase, both days (days 15–16) were used to calculate BP. The aforementioned days were chosen so that comparisons could be made between phases with different durations. For each of the phases, we report the single day in which the greatest number of individuals had BP measurements (see Results), as measures showed statistically significant reproducibility across days within a phase.

2.4. Statistical analyses

Analyses were performed with SPSS 11.5 for Windows (SPSS Inc., Chicago, IL, USA) or SAS 9.1 statistical software package (SAS Institute Inc., Cary, NC, USA) using PROC MIXED. A one-way, mixed-model analysis of covariance (ANCOVA) with line type (HR vs. C mice) as the main effect was used for the analyses of BP measures during both the baseline and wheel access phases. The withdrawal phase was analyzed as separate one-way ANCOVAs for mice in the wheel-blocked and wheel-free conditions, respectively. Additionally, a repeated-measures analysis was conducted across the three sampling phases. All animals were included in the repeated-measures analyses (even animals that did not have measurements from every sample phase). In all of the models, body mass, body temperature, and age were used as covariates. Batch and the presence/absence of the mini-muscle phenotype were tested as cofactors, but batch was never significant, and was therefore removed from the final analyses. The mixed-model ANCOVA used in these analyses includes line type as a fixed effect with line as a random effect nested within line type; degrees of freedom for testing the effects of line type, wheel access, and their interaction are 1 and 6. Outliers were determined using a formal statistical test (see [63,64]). Variables were transformed as needed to improve normality of residuals from statistical models. All tests were 2-tailed, and statistical significance was taken as $P \leq 0.05$.

3. Results

Body mass did not differ statistically between line types when mice started ($P = 0.1991$) or ended ($P = 0.1773$) wheel access, but individuals expressing the mini-muscle phenotype ($N = 6$ from lab designation line #3; $N = 3$ from lab designation line #6) had a lower body mass than HR or C mice that did not express the phenotype at both instances ($P = 0.0179$ and 0.0026, respectively). Similar results held throughout the measurements and at dissection (Tables 1–3). HR mice had significantly lower body temperatures during the withdrawal phase in the Wheels free group (Table 2). Mini-muscle mice had lower temperatures during baseline measurements (Table 1), but not during measurements in the wheel-access or withdrawal phases (Tables 1, 2).

Wheel running data were analyzed from the last two days of the wheel access phase. As expected from numerous previous studies, HR mice had higher wheel revolutions than C mice ($P = 0.0267$), and the ratio of mean wheel revolutions (HR/C) was 2.8. Both mean revolutions per minute (RPM) and maximum RPM were higher in HR mice ($P = 0.0005$ and $P < 0.0001$, respectively), but the number of minutes run (square-root transformed) did not differ between HR and C mice ($P = 0.1889$). A separate set of analyses that included body mass as a covariate indicated that it was never a significant predictor of wheel running (results not shown). Mini-muscle individuals ran significantly more revolutions per day ($P = 0.0079$) and at higher average ($P = 0.0017$) and maximum ($P = 0.0107$) speeds, but not for greater duration ($P = 0.1968$).

Reliability analysis (SPSS Inc., Chicago, IL, USA) revealed that during the final three days (days 6–8) of baseline systolic blood pressure (intraclass correlation = 0.385, $P = 0.003$, $N = 19$), diastolic blood pressure (intraclass correlation coefficient = 0.340, $P = 0.008$, $N = 19$), and mean arterial pressure (intraclass correlation coefficient = 0.352, $P = 0.007$) were statistically significantly reliable. For the final three days (days 12–14) of the wheel access phase ($N = 22$) reliability analysis revealed that systolic blood pressure

Table 1
Significance levels for blood pressure measurements during baseline and wheel-access periods.

Trait and transform	N	$P_{Selection}$	$P_{Mini\text{-}muscle}$	$P_{Bodymass}$[a]
Baseline				
Body mass (g)	71	0.1459 −	**0.0139** −	
Body temperature	69	0.0772 −	**0.0469** −	0.8629 +
Heart rate, log_{10}	51[b]	0.7699 −	0.3801 −	0.1637 −
Systolic BP, log_{10}	53	0.3693 +	0.0643 +	**0.0094** +
Diastolic BP, log_{10}	52[b]	0.2683 +	0.0899 +	**0.0361** +
Mean arterial pressure, log_{10}	53	0.3044 +	0.0615 +	**0.0183** +
Wheel access				
Body mass (g)	67[b]	0.1437 −	**0.0074** −	
Body temperature	66	0.1288 −	0.3980 −	0.6860 +
Heart rate, log_{10}	35[b]	0.9653 +	0.5228-	0.9788 +
Systolic BP, log_{10}	36	0.8458 −	0.8293-	0.1458 +
Diastolic BP, log_{10}	36	0.4166 −	0.7990 +	0.5831 +
Mean arterial pressure, log_{10}	36	0.5369 −	0.9097 +	0.3745 +

Significance values reported in this table (bold indicates $P \leq 0.05$) are from one-way ANCOVAs with line type as the main effect and presence of the mini-muscle phenotype, body mass, body temperature, and age as covariates or cofactors.
Baseline significance values are from day 7 only of the 8-day baseline measurement period. Wheel access significance values are from day 6 only of the 6-day wheel access period.
Age was used as a covariate in all analyses (results not shown).
Selection: + indicates that mice from high-runner lines have larger measurements, − indicates smaller measurements.
Mini-muscle: + indicates that mini-muscle mice have larger measurements, − indicates smaller measurements.
Body mass: + indicates positive association with body mass.
[a] Raw body mass used as a covariate.
[b] Indicates removal of one or more statistical outliers.

(intraclass correlation = 0.384, $P = 0.002$), diastolic blood pressure (intraclass correlation coefficient = 0.427, $P = 0.001$), and mean arterial pressure (intraclass correlation coefficient = 0.416, $P = 0.001$) were statistically significantly reliable. During the withdrawal phase (days 15–16) we assessed reliability in each of the two groups separately (continued wheel access vs. wheels blocked). For the wheel access group (N = 8) reliability analyses revealed that no measure was statistically significant (intraclass correlation coefficient <0.08, $P > 0.4$). However,

Table 2
Significance levels for blood pressure measurements during the withdrawal phase for mice with wheels blocked and wheels free, respectively.

Trait and transform	N	$P_{Selection}$	$P_{Mini\text{-}muscle}$	$P_{Bodymass}$[a]
Wheels blocked				
Body mass (g)	36	0.2416 −	**0.0153**-	
Body temperature, log_{10}	35[b]	0.6933 −	0.0514 −	0.7976 +
Heart rate	19[b]	0.3564 −	0.4545-	0.4438 +
Systolic BP, $\times^3$	19[b]	**0.0268** −	0.7324 −	0.0991 −
Diastolic BP, $\times^3$	20	**0.0271** −	0.6993 −	0.1621 −
Mean arterial pressure, $\times^3$	20	**0.0302** −	0.9204 −	0.2340 −
Wheels free				
Body mass (g)	32	0.1469 −	0.0519 −	
Body temperature, log_{10}	30	**0.0246** −	0.4814 +	0.3452 +
Heart rate	12	0.4673 −	0.9058 −	0.6841 +
Systolic BP	12	0.5243 +	0.6961 −	0.5828 −
Diastolic BP	12	0.3751 +	0.7296 −	0.5252 −
Mean arterial pressure	12	0.4131 +	0.7234 −	0.5335 −

Significance values reported in this table (bold indicates $P \leq 0.05$) are from separate one-way ANCOVAs with line type as the main effect and presence of the mini-muscle phenotype, body mass, body temperature, and age as covariates or cofactors.
Significance levels are from day 2 only of the 2-day withdrawal period.
Age was used as a covariate in all analyses (results not shown).
Selection: + indicates that mice from high-runner lines have larger measurements, − indicates smaller measurements.
Mini-muscle: + indicates that mini-muscle mice have larger measurements, − indicates smaller measurements.
Body mass: + indicates positive association with body mass.
[a] Raw body mass used as a covariate.
[b] Indicates removal of one or more statistical outliers.

Table 3
Significance levels for body and organ masses at dissection.

Trait and transform	N	$P_{Selection}$	$P_{Mini\text{-}muscle}$	$P_{Bodymass}$
Body mass (g)	68	0.1222 −	**0.0004** −	
Body mass (g)	68	0.2957 −	**0.0001** −	<**.0001** +[a]
Snout rump length (mm)	68	0.1807 −	0.2247 −	
Tail Length (mm)[b]	67[c]	0.7111 +	0.9349 −	
Ventricle mass, log_{10} (g)	68	**0.0412** +	**0.0004** +	<**.0001** +
	68	**0.0403** +	<**.0001** +	<**.0001** +[d]
Spleen mass, log_{10} (g)	67[c]	0.2542 +	0.2868 +	**0.0003** +
		0.3465 +	0.2529 +	**0.0005** +[d]

Significance values reported in this table (bold indicates $P \leq 0.05$) are from one-way ANCOVAs with line type as the main effect and presence of the mini-muscle phenotype, body mass and age as covariates or cofactors.
Age was used as a covariate in all analyses (results not shown).
Selection: + indicates that mice from high-runner lines have larger measurements, − indicates smaller measurements.
Mini-muscle: + that indicates mini-muscle mice have larger measurements, - indicates smaller measurements.
Body mass: + indicates positive association with body mass.
[a] Snout rump length as the covariate for body mass.
[b] Tail length = total length - snout-rump length.
[c] Indicates removal of one or more statistical outliers.
[d] Log_{10} body mass used as a covariate (unless indicated by [d], raw body mass was used as covariate).

there was one outlier, and given the small sample size it proved to be very influential on the overall results (see Supplemental Fig. 1A–C). Upon removal of this individual all measures were significantly reliable (intraclass correlation coefficient >0.6, $P < 0.03$). For the no-wheel group (N = 12) reliability analyses revealed that no measure was statistically significant (intraclass correlation coefficient >0.25, $P > 0.4$). Here again, there appeared to two influential data points which may disproportionately affect the correlation coefficients given the small sample size (see Supplemental Figs. 2A–C).

Given the strict inclusion criteria we imposed on our data set, we report measurements from the days with the highest sample sizes only (baseline day 7, wheel access day 6, and withdrawal day 2) (Tables 1, 2). HR and C mice did not differ statistically for any BP measurement during baseline or wheel access phases (Table 1), and heart rate was not significantly different between the line types at any phase (Tables 1, 2).

When the three sample phases were analyzed together in a repeated-measures design (N = 34 mice), all mice showed a statistically significant increase in DBP ($P = 0.0240$) and MAP ($P = 0.0355$), but not SBP ($P = 0.0917$) across the sampling phases (Fig. 2), but there was no statistically significant interaction between sample phase and line type for DBP, MAP, or SBP ($P = 0.0888$, $P = 0.1072$, and $P = 0.1706$, respectively). However, in a separate analysis of the wheel-blocked mice from the withdrawal phase, the HR mice had a lower SBP, DBP and MAP than C mice (Table 2 and Fig. 2). Heart rate did not differ significantly across phases in a repeated-measures analysis (results not shown).

Adjusting for variation in body mass, ventricle mass was larger in HR mice and in mini-muscle mice (Table 3). Neither spleen mass nor body length differed between line types or with regard to the presence/absence of the mini-muscle trait.

4. Discussion

To our knowledge, this is the first time the physiological effects of exercise withdrawal have been studied in mice. The BP inclusion criteria used in the present study were conservative, so the physiological effects reported here may underestimate the cardiovascular response to exercise withdrawal. Many previous studies using indirect BP measurements via tail cuff methodology have neglected to account for confounding variables (e.g., heart rate in excess of 900 beats/min) that are known to invalidate BP measurements (e.g., [57,60,65–67]). We individually evaluated each measurement cycle and used multiple inclusion criteria

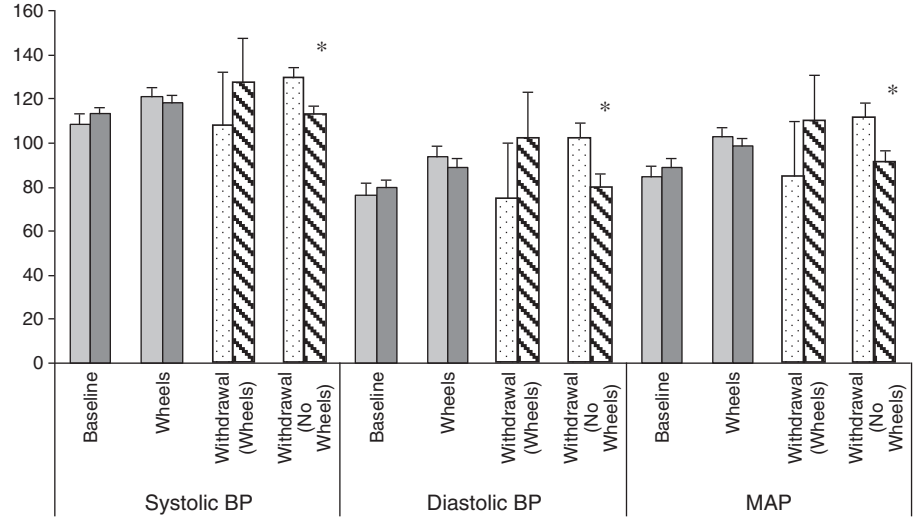

Fig. 2. Blood pressure (BP) measurements during baseline, wheel access, and withdrawal phases. Solid bars represent HR (dark) and C (light) mice during baseline and wheel access phases. Textured bars represent HR (hashed) and C (stippled) mice during the withdrawal phase, when the sample was split so that half the mice had their wheel access blocked ("No Wheels") and half of the mice were allowed continued wheel access ("Wheels"). Values for the baseline and wheel access phases are least squares means with standard errors from two-way ANCOVAs (line type × wheel access) with covariates of body mass, body temperature, and age, as well as presence of the mini-muscle phenotype as a cofactor. Values for the withdrawal phase are least squares means with standard errors from one-way ANCOVAs (line type) with the same covariates and cofactor. The asterisks indicate a significant ($P \leq 0.05$) line type difference for SBP, DBP, and MAP (see Table 2) during the withdrawal phase (HR < C mice). The differences across phases (baseline, wheel access, and withdrawal) were significant in repeated-measures ANCOVAs (SBP, $P = 0.0005$; DBP, $P = 0.0006$; MAP, $P = 0.0005$).

(heart rate, tail blood volume, pressure curves) in selecting BP measurements.

The present study is the first examination of BP in the High Runner lines of mice, and therefore a secondary finding involving BP in the mini-muscle mice, which represent a subset of the HR phenotype [49,50], is also reported here. We hypothesized previously [52] that mini-muscle mice would have higher BP values both because of their larger ventricle mass (Table 3) and because of their smaller muscle mass that has the same whole-animal metabolic rate per gram of body mass [49] and presumably requires more blood per gram to adequately perfuse the muscle, thus elevating BP. Additionally, the finding that mass-specific aerobic capacity [51] and muscle capillarity is higher in mini-muscle mice seems consistent with a higher oxygen demand and greater blood flow per gram of muscle tissue [54]. However, BP did not differ significantly between mini-muscle mice and other mice during any of the phases of the present study. There was a trend at baseline, however, for a higher resting BP ($P < 0.1$ for SBP, DBP, and MAP: Table 1) in mini-muscle mice. Given the increased ventricular mass of the heart, peripheral resistance may play a larger role in cardiovascular dynamics "at rest" in these mice. Interestingly, this trend disappeared during both the wheel-access and withdrawal periods, which suggests that increased stroke volume or enhanced peripheral vasodilation may be compensating for greater peripheral resistance during these phases. However, given that we measured BP during the day (i.e., the inactive period for these nocturnal animals), what BP does during voluntary exercise on wheels in mini-muscle mice remains an open question.

Only a few studies in humans have examined the physiological effects of exercise withdrawal [1,6], and these studies have not measured BP. However, alterations in BP are known to be a key component in physiological withdrawal from addictive drugs [15,18,19,22,25–32]. A wide variety of withdrawal syndromes have been defined, and the neurobiology of many chemical and behavioral dependencies has been characterized (reviewed in [68]). Despite this work, the suite of physiological events in exercise withdrawal has not been systematically explored. Exercise is a self-rewarding behavior in rodents [37,38], and multiple generations of selection have increased voluntary wheel running in HR mice by nearly 3-fold (e.g., [41,49]). Consequently, the lower BP (SBP, DBP, and MAP) observed in HR mice with wheels blocked (Table 2, Fig. 2) is indicative of an altered physiological state that is consistent with withdrawal (e.g., similar to the lower SBP in nicotine withdrawal). In withdrawal syndromes, an alteration in BP commonly occurs following the cessation of the dependent substance/activity, along with a suite of other behavioral and physiological responses. We do acknowledge that these physiological responses (especially in response to short-term dependency) may or may not be indicative of centrally mediated withdrawal, but alternatively may be indicative of acute phenotypic flexibility (i.e., a return to "normal" physiological function). We saw no evidence of this in the current investigation, as indicated by a lack of differential (between HR and C mice) acute effects of wheel running on systolic BP, diastolic BP, and MAP as shown in Table 1/Fig. 2. Thus, although this does not definitively rule out a physiological effect in the absence of a central mechanism, we do believe that prior evidence strongly implicates a neurobiological underpinning (see Ref. [45]). In a previous study in HR mice, neural alterations were observed following wheel deprivation that involved heightened activity in brain regions devoted to anxiety and reward [47]. Additionally, HR mice have elevated levels of basal corticosterone [69,70], which over time can have deleterious effects, including predisposition to affective disorders [71]. Malisch et al. [16] found evidence of increased depressive-like behavior in HR mice, as measured by greater time spent immobilized in forced-swim trials. These differences in neural, behavioral, and now cardiovascular responses to wheel-running deprivation, when taken together, suggest a physiological withdrawal response, coupled with an evolved exercise dependency in these HR mice.

In contrast to physiological withdrawal, the behavioral and psychosocial effects of exercise withdrawal have been well characterized in humans [1,3,72–75]. However, these qualitative assessments (e.g. mood, irritability) are difficult to translate more broadly across species as a distinct behavioral withdrawal syndrome.

In trying to model components of behavioral addictions, Brown [76] proposed six components developed from studies of gambling addiction in humans: salience, euphoria, tolerance, withdrawal symptoms, conflict, and relapse. Applying these human components to addictions in other species is problematic. Conflict was defined as "interpersonal conflict" resulting from a loss of sociality whilst pursuing the addictive activity, which arguably has little or no relevance for mice

housed individually with running wheels. Of the remaining components, euphoria and tolerance have not been directly tested in these mice. However, HR mice readily resume high levels of wheel running when wheel access has been previously denied (i.e., they "relapse": unpublished results). Moreover, evidence for salience, defined as the perceived value of the activity, has been suggested by behavioral studies [77]. Combined with the finding in the present study of a physiological withdrawal response, three components of Brown's [76] model of behavioral addiction are supported in HR mice. Further work examining these remaining components could lead to a conclusive understanding of the apparent exercise addiction in these mice.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.physbeh.2013.02.010.

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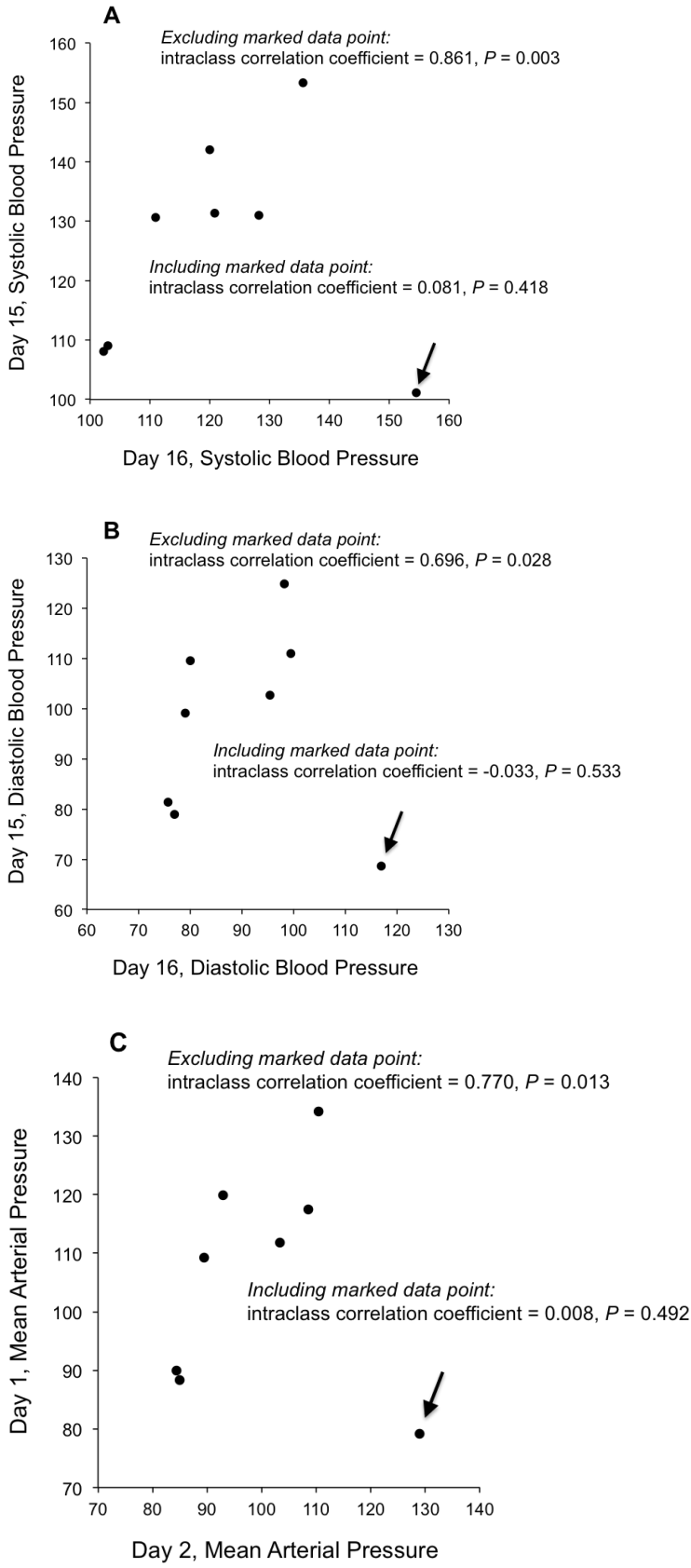
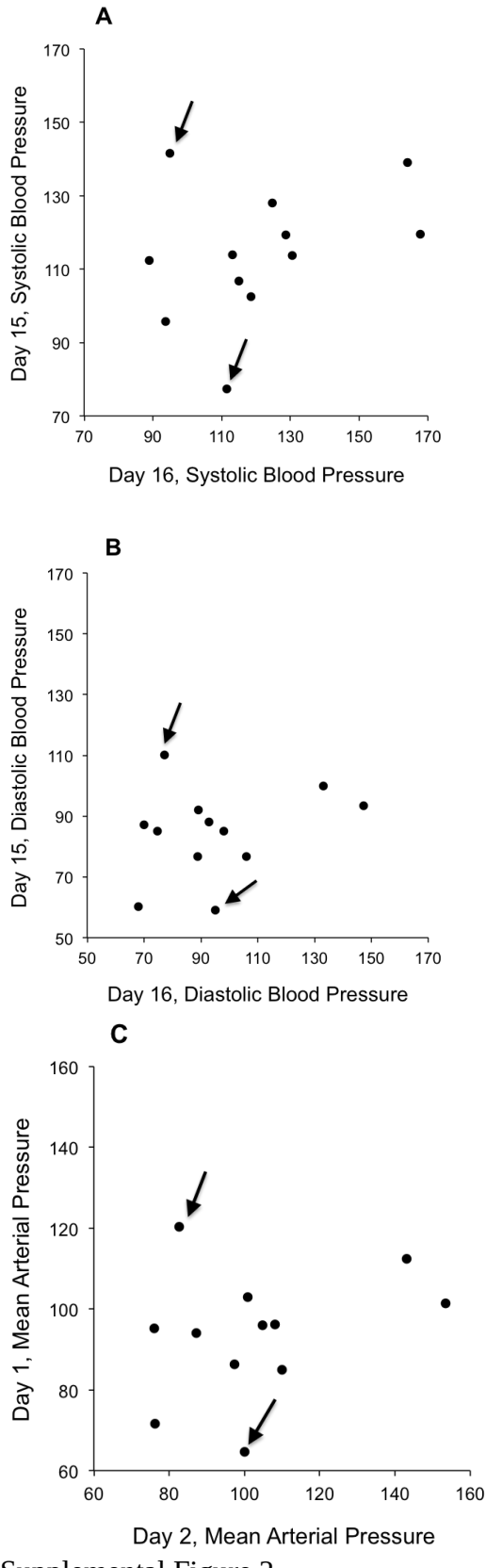


Supplemental Figure 1



Supplemental Figure 2