

Behavioral Despair and Home-Cage Activity in Mice with Chronically Elevated Baseline Corticosterone Concentrations

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Abstract Dysfunction of the hypothalamic-pituitary-adrenal axis resulting in elevated baseline glucocorticoid concentrations is a hallmark of stress-related human anxiety and affective disorders, including depression. Mice from four replicate lines bred for high voluntary wheel running (HR lines) run almost three times as much as four non-selected control (C) lines, and exhibit two fold elevated baseline circulating corticosterone levels throughout the 24 h cycle. Although elevated baseline CORT may be beneficial for high locomotor activity, chronic elevations can have deleterious effects on multiple systems, and may predispose for affective disorders. Because stressful events often precede a depressive bout, we quantified depressive-like behavior in the forced-swim (FST; generation 41) and tail-suspension tests (TST; generation 47) in HR and C mice that had wheel access for 6 days and then were deprived of wheels on day seven prior to the FST or TST. Male HR spent significantly more time immobile in the FST than C, suggesting that HR males have a predisposition for depression-like behavior. Both male and female HR

(generation 43) were more active than same-sex controls in both wheel running and home-cage activity across 22 h (pooling the sexes, HR/C = 2.28 and 2.66, respectively).

Keywords Artificial selection · Despair behaviors · Exercise · Experimental evolution · Glucocorticoids · Hyperactivity · Wheel running

Introduction

Dysregulation (both hyper- and hypo-activation) of the hypothalamic-pituitary-adrenal (HPA) axis is associated with various human neuropsychiatric disorders, including depression (Parker et al. 2003), anxiety (Strohle and Holsboer 2003), attention-deficit hyperactivity disorder (Kaneko et al. 1993), and post-traumatic stress disorder (Yehuda et al. 1990). Chronic activation is associated with anxiety and affective disorders, including panic disorder and major depression (Parker et al. 2003; Sapolsky and Plotsky 1990; Strohle and Holsboer 2003). Elevated baseline secretion of glucocorticoids is seen in over 50% of depressed patients, and some investigators have suggested that HPA dysregulation should be considered a main element of the clinical phenotype (Strohle and Holsboer 2003). Additionally, hypercortisolism may be involved in the etiology of depression [e.g., normalization of HPA dysregulation is often necessary for clinical remission of affective disorders (Holsboer 2000); see also (Barden et al. 1995)].

Hypercortisolism in people with affective disorders has been attributed to multiple changes within the HPA axis (Amsterdam et al. 1989; Gold et al. 1986). Because depressed patients typically fail to suppress endogenous glucocorticoids when high levels of synthetic glucocorticoids (e.g., dexamethasone) are administered, decreased

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negative feedback sensitivity is implicated. Further studies have implicated changes in glucocorticoid (GR) and mineralocorticoid (MR) receptors in the brain (particularly in the hippocampus) as the mechanism (de Kloet et al. 1998; Holsboer 2000; Young et al. 2003). Some benefits of antidepressant medications act through GR and MR to normalize the HPA axis (Holsboer 2000). Furthermore, mice with reduced GR in the forebrain have elevated baseline CORT, impaired negative feedback within the HPA axis, and increased “depression-like behaviors” (Boyle et al. 2005).

Mice selectively bred for high voluntary wheel running (Garland 2003; Rhodes et al. 2005; Swallow et al. 1998, 1999) may provide insights into the neuroendocrine basis of anxiety and affective disorders. The four replicate High Runner (HR) lines have circulating CORT levels that are elevated across the circadian cycle and are on average two-fold higher than the four non-selected control (C) lines (Girard and Garland 2002; Malisch et al. 2007, 2008), which should predispose them to depressive behavior. However, HR lines are almost three times as active as C, and exercise is typically associated with amelioration of depression and has both anxiolytic and antidepressant effects in humans (Dunn et al. 2005; Morgan 1985; Ransford 1982). In rats and mice, voluntary wheel running has numerous positive effects at many levels of biological organization, from behavioral to cell processes (see Dishman et al. 2006 for review), including reducing learned helplessness (i.e., behavioral depression) (Greenwood et al. 2003, 2005; Duman et al. 2008). When wheel access is blocked, HR lines show a neurobiological profile (based on cFos imaging of neuronal activity) that resembles narcotic withdrawal (Rhodes et al. 2003, 2005). In somewhat similar fashion, human competitive runners deprived of their scheduled training report significant withdrawal-like symptoms, including depressed mood and elevated resting heart rate (Aidman and Woollard 2003).

Two assays of depression-like behavior are commonly used in rodents, the forced-swim test (FST; Porsolt et al. 1977) and the tail-suspension test (TST; Steru et al. 1985). Reactions to the FST and TST may have different neuroendocrine bases (see “Discussion”). Therefore, we applied both the FST and TST to HR and C mice. The onset of a human depressive episode is often associated with major life stress (Brown et al. 1987; Dunner et al. 1979; Hammen et al. 1992; Post 1962), and genetically modified mice with altered glucocorticoid receptor expression in the CNS, a proposed mouse model of depression, only exhibit depression-like behavior following acute stressors (Ridder et al. 2005). To mimic stressful life events, we administered the FST and TST to HR and C mice that had wheel access for 6 days and were then deprived of wheels on day seven, prior to the FST or TST. A previous study found that such wheel deprivation can cause major changes in the activity of

certain brain regions, with differential effects in the HR and C lines (Rhodes et al. 2003, 2005), suggesting that HR lines are frustrated and/or stressed when wheel access is denied. We also measured activity levels in mice housed in standard cages without wheels. We hypothesized that HR mice would be more active than C both in home-cages and wheels, and would spend more time immobile in the FST and TST when “stressed” by denying wheel access.

Materials and methods

Study animals

The artificial selection experiment began in 1993 from a base population of outbred Hsd:ICR mice. The selection criterion was total revolutions run on days 5 + 6 of a 6 day exposure to a Wahman-type activity wheel (1.12 m circumference; Lafayette Instruments, Lafayette, IN, USA) when mice were ~6–8 weeks of age (Swallow et al. 1998; Garland 2003; Rhodes et al. 2005). Four replicate lines were bred for high running (HR lines), and four replicate non-selected lines were maintained as controls (C lines). At weaning, mice were toe-clipped for identification and housed randomly in same-sex groups of four. During the entire selection experiment and throughout this study, mice were maintained on a 12:12 h light:dark cycle with lights on at 0700 h, and were provided with food and water ad libitum. All animals used in these experiments were housed and maintained in accordance with NIH animal care guidelines, and all procedures were approved by the IACUC of the University of California, Riverside, an AAALAC-accredited institution.

Wheel-running activity

Wheel running was recorded as part of the routine selection protocol during the forty-third generation of selection. In brief, 7- to 9-week-old mice ($N = 78$ C line females, $N = 79$ C line males, $N = 117$ HR line females, and $N = 112$ HR line males) were allowed wheel access for 6 days. All data presented here were collected on the 6th day of wheel access. Wheel running was measured in four batches of ~100 mice per week, for four consecutive weeks. Wheels were attached to standard housing cages (see Swallow et al. 1998), and running was recorded as the total number of revolutions in 1 min blocks for 23 h (1300–1200 h) by an automated system (San Diego Instruments, San Diego, CA, USA).

Home-cage activity

Home-cage activity was measured in adult (10- to 12-week-old) males and females that were siblings of the wheel

running mice. All individuals ($N = 96$, six males and six females from each line) were obtained from different families. As described in Malisch et al. (2008), activity was measured using four motion and activity detector units (MAD-1: Sable Systems[®]) interfaced with Warthog Labhelper software (Warthog, <http://www.warthog.ucr.edu>). Mice were housed individually 5 days prior to measurement to mimic housing conditions during the wheel-testing protocol. On day six, each cage was placed, with as little disturbance as possible, on a MAD-1. Activity was recorded twice per second, from 1100 until 1000 h the following morning (23 h) using Labhelper Software. Data were converted to normalized activity units (NAU; for complete data reduction procedures, see Malisch et al. 2008). In brief, we computed NAUs by converting baseline-corrected files (which included positive and negative voltages) into absolute values, normalizing by dividing by the mean of the lowest consecutive 10 min (presumed to be sleep), and then subtracting 1.0 from the result. Thus, a NAU of zero indicates presumptive sleep, and higher numbers indicate proportionately scaled activity intensity (e.g., a NAU of 4 indicates twice the activity-related force generation of a NAU of 2).

Forced-swim test

Forced-swim behavior was measured in 6–8-week-old mice from generation 41. One male and one female were chosen at random from each of six different families per line (total $N = 96$, i.e., six per sex from each of the four HR and four C lines). At ~ 6 weeks of age, mice were housed singly with wheel access for 6 days. Wheel access was blocked at 1200 h and the FST was administered between 2100 and 0200 h (i.e., during scotophase) under red light illumination.

We used the procedure of Porsolt et al. (1977) for the FST. Individual mice were placed in a glass cylinder (15 cm diameter) containing 10 cm of water at 25°C. Mice were allowed to acclimate to the testing apparatus for 2 min, then their activity was continually monitored for four additional minutes. The amount of time (in seconds) spent immobile in a floating posture with only minimal movements necessary to stay afloat was recorded by one of two trained observers.

Tail-suspension test

Tail-suspension behavior was measured in 6–8-week-old mice from generation 47. One male and one female were chosen at random from each of six different families per line ($N = 96$, six per sex from each of the four HR and four C lines). At ~ 6 weeks of age, mice were housed singly with wheel access for 6 days. Wheel access was blocked at 1200 h and the TST was administered between 2100

and 0200 h (i.e., during scotophase) under red light illumination.

Mice were suspended by their tails from a padded plastic clip, in turn suspended from a ring stand. The ring stand was placed on the middle of a motion and activity detector unit (MAD-1: Sable Systems[®]) interfaced to a Macintosh computer equipped with an A-D converter and Labhelper software. The MAD-1 transduces activity as voltage, with signal intensity magnitude correlated to activity intensity. Activity was recorded ten times per second for a total of 6 min. Labanalyst software (Warthog Systems) was used to eliminate electrical drift using baseline correction.

Statistics

Graphs of wheel-running activity and home-cage activity were constructed using simple means \pm SE for 20 min time blocks from 1300 to 1100 h (a 22 h period where both behavioral assays overlap). Analyses for males were previously reported in Malisch et al. (2008). Here, we examine both males and females in a combined statistical model. Statistical differences were assessed for total activity (summation of 22 h) as well as nine 2-h blocks. The 2-h blocks included: the first 6 h (1300–1500 h; 1500–1700 h; 1700–1900 h) when lights were on and mice are typically inactive; 8 h following lights out, with the first 20 min bin excluded (see Malisch et al. 2008, 1920–2120 h; 2120–2320 h; 2320–0120 h; 0120–320 h); and the 4 h following lights on (0700–0900 h; 0900–1100 h).

We used a two-way mixed-model analysis of covariance (ANCOVA) with Type III tests of fixed effects in SAS Procedure Mixed (SAS Institute, Cary, NC, USA). Primary fixed effects were sex and linetype, replicate lines were a random effect nested within linetype, and family was nested within line. In such an analysis, the effect of linetype is tested relative to the variance among lines, with 1 and 6 degrees of freedom. Inclusion of all eight lines (4 selected, 4 control) is necessary because lines can and do diverge by random genetic drift. Thus, a comparison of any one selected line with any one control line may reveal many differences that have nothing to do with the selection protocol per se and so represent spurious “correlated responses” to selection (e.g., see Henderson 1989, 1997; Garland et al. 2002; Garland and Rose 2009).

Testing age was included as a covariate, and models were run both with and without body mass as an additional covariate because mice from the HR lines are smaller than C (Swallow et al. 1999). For wheel-running analysis, family and measurement batch were additional random effects, and wheel freeness (an inverse measure of wheel resistance to being turned) was used as an additional covariate to control for variation among wheels. P values for wheel running and home-cage activity are 2-tailed, and statistical significance

for the 2 h blocks (but not total activity) was judged at $P < 0.0056$ (i.e., 0.05/9) as a maximally conservative adjustment for multiple comparisons. For activity and in all other analyses in this study, degrees of freedom for testing the linetype effect were 1 and 6.

Comparison of linetypes (HR vs. C) in the FST employed a one-way mixed-model analysis of variance (ANOVA). Linetype was a fixed effect; replicate lines were a random effect nested within linetype. The observer was included as a random cofactor. Because we had a directional hypothesis (HR should exhibit more floating), we employed a 1-tailed test. We had no a priori hypothesis concerning sex differences, so we analyzed the sexes separately.

Tail-suspension results were analyzed in two ways. First, we analyzed activity across the full 6 min test (sum of all 3,600 samples). Because there may be acclimation to the apparatus, we also analyzed total activity in the final 4 min of the 6 min test. As with the FST data, TST data were analyzed with a one-way mixed-model analysis of variance. Body mass is related to the force generated by locomotor activity; therefore, mass was used as a covariate. Although we again had a directional hypothesis (HR should exhibit less struggling), the difference was in the opposite direction, and so we do not report P values (e.g., see Sokal and Rohlf 1981). Again, we had no a priori hypothesis concerning sex differences in TST, and so did not test for a sex effect.

Results

Locomotor activity

When examined in 2 h blocks, neither wheel-running activity nor home-cage activity differed statistically between HR and C mice during the photophase (700–1900 h) in either sex (Tables 1, 2). Both HR and C mice showed a distinct onset of wheel running and home-cage activity at lights off (1900 h; Figs. 1, 2). During scotophase, HR mice were significantly more active than same-sex C animals for the 8 h following lights off (Fig. 1; Tables 1, 2). As previously reported (e.g., Swallow et al. 1998, 1999; Garland 2003; Rhodes et al. 2005), females tend to run more than males regardless of linetype (see Table 1; Fig. 1); however, the sex effect was not statistically significant after adjusting for multiple comparisons (i.e., P was not <0.0056). Sex did not significantly affect home-cage activity for any of the 2 h blocks. Neither body mass nor age consistently predicted either type of activity. Wheel freeness never had a significant effect.

Summing across 22 h (1300–1100 h), HR were more active than C for both wheel running ($P_{\text{linetype}} = 0.0002$) and log-transformed home-cage activity ($P_{\text{linetype}} = 0.0021$).

Females were more active than males in wheel running ($P_{\text{sex}} = 0.010$), but not home-cage activity ($P_{\text{sex}} = 0.635$). The sex \times linetype interaction was not significant for either wheel running or home-cage activity ($P = 0.191$ and 0.213 , respectively). The HR/C ratio of least squares means for total wheel revolutions was $9,923.5/4,349.0 = 2.28$. Separating by sex and linetype, LS means and standard errors for total wheel revolutions were: C males = $4,005.92 \pm 707.27$, C females = $4,692.14 \pm 708.32$, HR males = $9,123.69 \pm 681.10$, and HR females = $10,723.00 \pm 677.12$.

For total home-cage activity, we computed normalized values (for 158,400 data points; 2 samples per second for 22 h) for each mouse. The factorial difference between HR and C lines in least squares means for total NAU for home-cage activity (back transformed from \log_{10}) was $18,314/6,873 = 2.66$. Least squares means and standard errors for \log_{10} transformed NAUs were: C males = 5.89 ± 0.087 , C females = 5.78 ± 0.064 , HR males = 6.25 ± 0.063 , and HR females = 6.27 ± 0.083 .

Forced-swim test

For males, the amount of time spent floating was twofold greater for HR than for C mice (Fig. 3; 1-tailed $P = 0.0305$), but females did not differ.

Tail-suspension test

Although we predicted that mice from the HR lines would struggle less (i.e., exhibit increased immobility), they actually struggled more than mice from the C lines when considering all 6 min (see Malisch 2007) or only the final 4 min (Fig. 4). Therefore, we do not report P values.

Discussion

One objective of this study was to establish activity levels of HR and C mice when housed without wheel access. A 2.5- to 3-fold increase in wheel-running activity has been well documented in the HR lines (reviewed in Garland 2003; Rhodes et al. 2005; see also Gomes et al. 2009). In the present study, summing across 22 h (1300–1100 h), HR lines ran 2.28-fold farther than C lines (see Results and Fig. 1). For home-cage activity, the difference was 2.66-fold, which bolsters the use of these lines as a generalizable model for high activity levels or “hyperactivity” (see also Rhodes et al. 2001, 2005; Malisch et al. 2008). How the activity levels of HR mice compare with those of other strains that exhibit increased locomotor or exploratory behavior is not yet known. However, HR mice do exhibit substantially higher wheel running than the inbred strain

Table 1 Significance levels (2-tailed *P* values) from analyses of wheel-running activity (revolutions) during nine 2-h blocks

Time	<i>N</i>	Trans-form	Linetype	Sex	Sex × linetype	Age	Wheel freeness
1300–1500	386	Rank	0.304	0.694	0.092	0.578	0.544
1500–1700	386	Rank	0.480	0.753	0.938	0.192	0.530
1700–1900	386	Rank	0.300	0.310	0.347	0.579	0.106
1920–2120	386	None	0.006	0.044	0.212	0.085	0.243
2120–2320	386	None	0.001	0.028	0.143	0.202	0.110
2320–0120	386	None	0.002	0.024	0.065	0.426	0.486
0120–0320	386	None	0.002	0.901	0.845	0.379	0.442
0700–0900	386	Rank	0.020	0.025	0.416	0.995	0.877
0900–1100	386	Rank	0.093	0.165	0.505	0.820	0.980
1300–1100	386	None	<0.001	0.010	0.191	0.141	0.470

Main effects in the ANCOVA included linetype (High Runner vs. Control lines), sex, the sex-by-linetype interaction; covariates were age and wheel freeness. Analysis of total activity across 22 h is shown in the bottom row

Table 2 Significance levels (2-tailed *P* values) from analyses of home-cage activity

Time	<i>N</i>	Trans-form	Linetype	Sex	Sex × linetype	Age	Body mass
1300–1500	96	log ₁₀	0.503	0.127	0.073	0.105	0.028
1500–1700	96	log ₁₀	0.285	0.792	0.140	0.075	0.918
1700–1900	96	log ₁₀	0.866	0.591	0.036	0.367	0.749
1920–2120	96	log ₁₀	0.001	0.626	0.760	0.030	0.556
2120–2320	96	log ₁₀	0.002	0.361	0.605	0.533	0.435
2320–0120	96	log ₁₀	0.002	0.739	0.687	0.603	0.943
0120–0320	96	log ₁₀	0.006	0.985	0.436	0.448	0.422
0700–0900	96	log ₁₀	0.971	0.340	0.946	0.859	0.083
0900–1100	96	log ₁₀	0.332	0.787	0.380	0.325	0.355
1300–1100	96	log ₁₀	0.002	0.635	0.213	0.747	0.726

Main effects in the ANCOVA included linetype (High Runner vs. Control lines), sex, the sex-by-linetype interaction; covariates were age and body mass. Analyses of nine 2-h blocks are shown, followed by analysis of total activity across 22 h in the bottom row

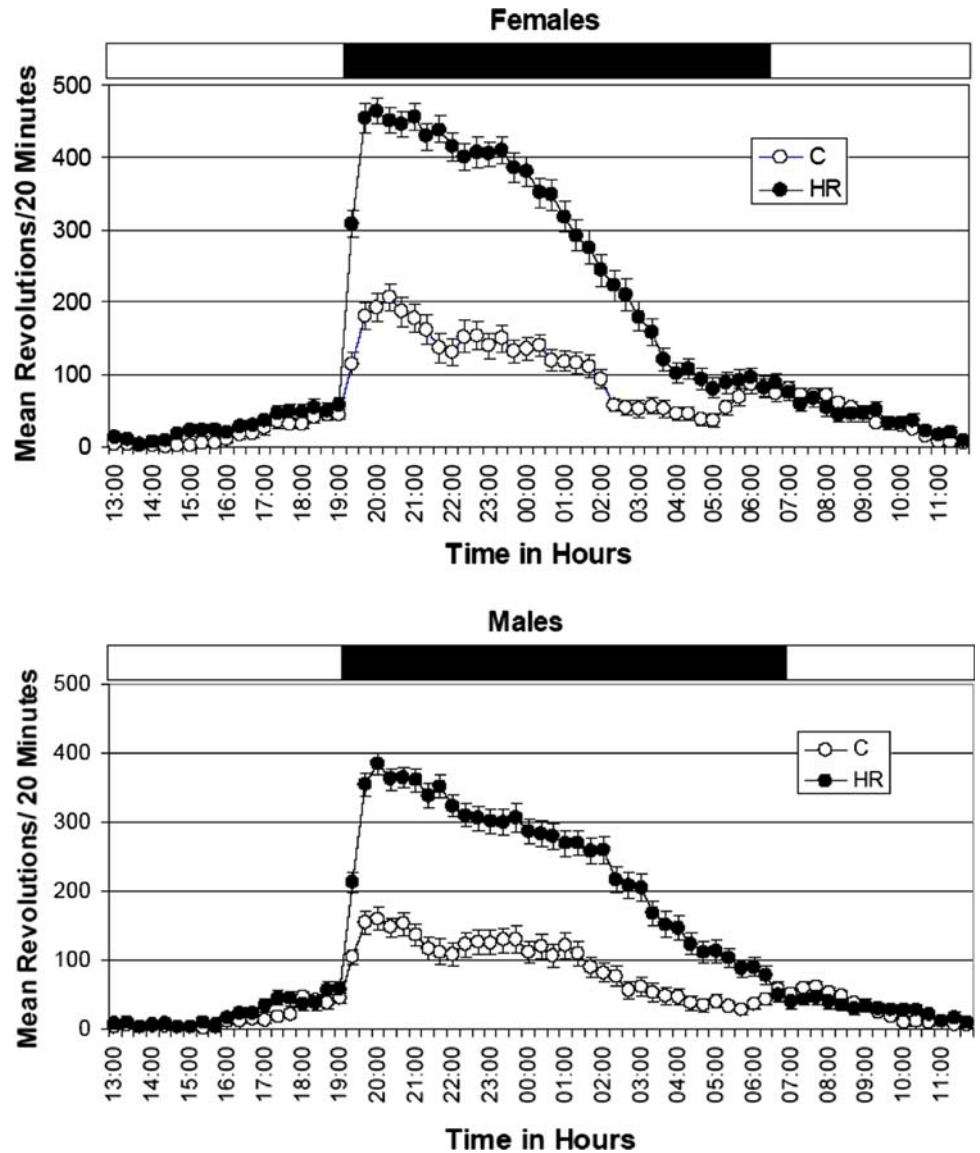
C57BL/6J or several other strains that had been selected for body mass and/or body fat (Nehrenberg et al. 2008).

As discussed elsewhere (Malisch et al. 2007, 2008), elevated CORT in the HR lines may promote wheel running by increasing available energy, increasing motivation to perform the behavior, or a combination of the two (Dallman et al. 1993; Pecoraro et al. 2006). However, elevated baseline CORT is associated with a number of deleterious consequences (e.g., on these lines of mice, see Malisch et al. 2007, 2009), and often accompanies the onset of depressive illness in human subjects (ratio of baseline CORT in psychotic depressives to control patients = 1.8; Board et al. 1956) as well as depression-like behavior in rodents. For example, chronic CORT elevation as seen in Cushing's disease and in patients taking chronic doses of therapeutic glucocorticoids are more likely to develop symptoms of depression (Antonijevic and Steiger 2003; Brown et al. 1999, 2004; Kelly et al. 1983). In both male and female rats, chronic exposure to exogenous CORT increases depressive behavior in a dose-dependent manner, as measured by the

FST (Gregus et al. 2005; Johnson et al. 2006; Kalynchuk et al. 2004). Male rats with a pharmacologically induced two fold elevation in baseline CORT (a similar difference as in HR versus C lines; average ratio of HR:C across six timepoints = 2.6; see Malisch et al. 2008), spent significantly more time immobile in the FST as compared with control rats (Johnson et al. 2006).

“Depression” is difficult to quantify in rodents, primarily due to the emotional nature of the symptoms (see Holmes (2003) for an insightful review of rodent models of depression). Two behavioral assays of depression-like behavior dominate the field, the forced-swim test (FST: Porsolt et al. 1977) and the tail-suspension test (TST: Steru et al. 1985). Both measure “behavioral despair”—the reaction to an adverse and inescapable situation (however, other interpretations have been made; see Holmes 2003). The time spent immobile, adopting a passive floating or hanging position, is measured. These tests were first employed to screen antidepressant drugs and have proven effective at predicting efficacy. The neuroendocrine bases

Fig. 1 Wheel-running activity during 20 min bins as a function of time since midnight. Values are simple means \pm SEs over 22 h (1300–1100). *Open circles* represent HR lines and *closed circles* represent Control lines. *Top panel* is for females ($N = 195$), *bottom* is for males ($N = 191$). Figure for males redrawn from Malisch et al. (2008). Lights were on from 0700 to 1900 shown as *open bars* above figure



of FST responses are not fully understood, and other tests for depressive phenotypes exist (see Holmes (2003) for a comprehensive list), but we were interested in these tests because injections of glucocorticoids increase immobility in rats (Johnson et al. 2006). Additionally, El Yacoubi et al. (2003) selectively bred mice for increased immobility in the TST and produced animals that not only have longer bouts of immobility but also exhibit other correlated traits symptomatic of depression, including elevated baseline CORT. Although both the FST and TST focus on immobility, marked differences in responses to the two tests have been observed both within and among strains of mice (Bai et al. 2001). Therefore, reactions to the FST and TST may differ in their neuroendocrine basis. In the present study, we used two assays to measure “behavioral despair,” but other tests to examine other depression-like symptoms in

rodents (e.g., the sucrose preference test to assess anhedonia, examination of sleep patterns) are clearly of interest for future studies.

Because of the association between elevated baseline CORT and depression-like behavior, we hypothesized that wheel-deprived HR lines would spend more time immobile in both the forced-swim and tail-suspension tests. As predicted, males from the HR lines spent significantly more time floating than C lines, but females showed little difference (Fig. 3). Because running is a rewarding behavior (Belke and Garland 2007; Brené et al. 2007) and elevation in plasma CORT increases the reward value of some behaviors (Pecoraro et al. 2005; Pecoraro et al. 2006), being deprived of wheels following 6 days of wheel access is likely stressful, particularly for HR mice (see Aidman and Woollard 2003; Rhodes et al. 2003, 2005). Increased

Fig. 2 Home-cage activity units in 20 min bins as a function of time since midnight. Values are simple means \pm SEs for total normalized activity (see text) over 22 h (1300–1100). *Open circles* represent C lines and *closed circles* represent HR lines. *Top panel* is for 48 females, *bottom* for 48 males. Figure for males redrawn from Malisch et al. (2008). Lights were on from 0700 to 1900 as shown by *open bars* above figure

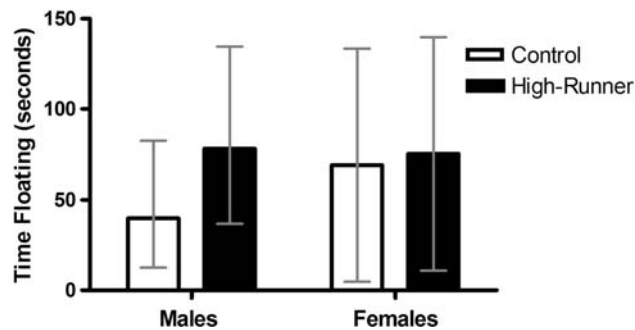
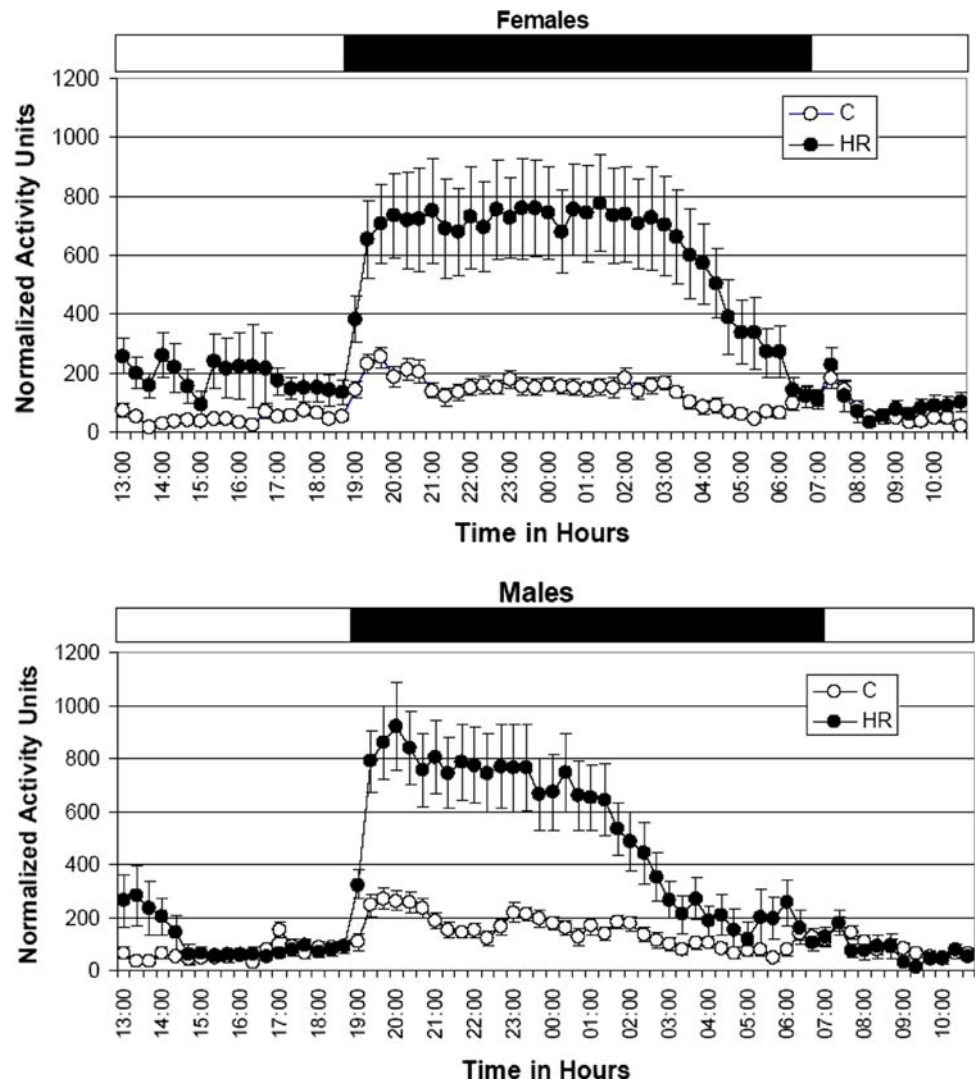


Fig. 3 Time spent floating in minutes during the forced-swim test. *Bars* represent adjusted least squares means (from one-way nested ANCOVAs) with 95% confidence intervals. Values for males have been back transformed from the square root scale. Male HR and C differ significantly (1-tailed $P = 0.0305$)

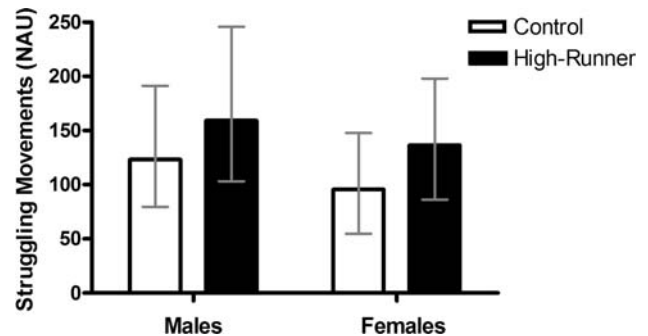


Fig. 4 Struggling behavior in normalized activity units during the tail-suspension test. *Bars* represent adjusted least squares means (from one-way nested ANCOVAs) with 95% confidence intervals for only the final 4 min of a 6 min test. Values for males have been back transformed from log base 10 scale and values for females have been back transformed from the square root scale

immobility in HR males following wheel deprivation suggests that elevated CORT in HR lines predisposes them to depression-like behavior.

Interestingly, we did not observe a difference between HR and C lines in another measure of depression-like behavior, immobility in the tail-suspension test (TST).

Although the amount of immobility in the FST and TST are positively correlated in some strains, and mice selectively bred for immobility in the FST are also less active in the TST (El Yacoubi et al. 2003), these tests do not always yield concordant results. Marked differences for depressive-like behavior have been documented both within and among strains (Bai et al. 2001), suggesting that immobility in the FST and TST may differ in their neuroendocrine basis. Furthermore, in a recent study by Stone and Lin (2008), experimentally elevated baseline CORT significantly increased mobility (anti-immobility) during the FST but did not significantly affect mobility in the TST. Stone and Lin (2008) report a trend for increased mobility in the TST with increasing CORT level, suggesting that swimming behavior during the FST may be more sensitive to exogenous CORT than struggling behavior in the TST (Stone and Lin 2008).

Voluntary exercise in human beings and rodents has numerous positive effects on cognition, neurotrophic effects, and reduces some negative consequences of stress (reviewed in Dishman et al. 2006). Human voluntary exercise has antidepressant effects (Dunn et al. 2005; Morgan 1985; Ransford 1982). Rodent voluntary exercise also has antidepressant-like effects; for example, voluntary wheel running has been shown to reverse stress-induced learning deficits in shuttle box escape behavior (Greenwood et al. 2005), to reverse behavioral consequences resulting from chronic, unpredictable stress (Zheng et al. 2006), and to reduce helpless behavior as measured by the FST (Duman et al. 2008). Because both exercise and chronic antidepressant treatment increase hippocampal brain-derived neurotrophic factor (BDNF), BDNF has been hypothesized to mediate the antidepressant effects of activity (see Duman and Monteggia 2006; Duman et al. 2008). In fact, in the study by Duman et al. (2008), when the BDNF-MAPK pathway was blocked, the antidepressant benefits of voluntary exercise were abolished. Previous research has shown that, with 7 days of wheel access, HR mice have higher levels of hippocampal BDNF than C mice also housed with wheels (Johnson et al. 2003). An interesting finding in the present study is that although the HR mice had 6 days for wheel access prior to wheels being blocked, HR mice still had higher levels of behavioral despair than C lines. These findings suggest that the “stress” of wheel deprivation overrides any potential BDNF benefit from the previous 6 days of continuous wheel access, a likely scenario because stress has been shown to reduce BDNF levels (for review see Duman and Monteggia 2006). Future studies of the HR and C lines should examine forced-swim behavior in the HR and C mice when both are allowed free wheel access without wheel deprivation.

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References

- Aidman EV, Woollard S (2003) The influence of self-reported exercise addiction on acute emotional and physiological responses to brief exercise deprivation. *Psychol Sport Exerc* 4:225–236. doi:10.1016/S1469-0292(02)00003-1
- Amsterdam JD, Maislin G, Gold P, Winokur A (1989) The assessment of abnormalities in hormonal responsiveness at multiple levels of the hypothalamic-pituitary-adrenocortical axis in depressive illness. *Psychoneuroendocrinology* 14:43–62. doi:10.1016/0306-4530(89)90055-3
- Antonijevic IA, Steiger A (2003) Depression-like changes of the sleep-EEG during high dose corticosteroid treatment in patients with multiple sclerosis. *Psychoneuroendocrinology* 28:780–795. doi:10.1016/S0306-4530(02)00085-9
- Bai F, Li X, Clay M, Lindstrom T, Skolnick P (2001) Intra- and interstrain differences in models of “Behavioral despair”. *Pharmacol Biochem Behav* 70:187–192. doi:10.1016/S0091-3057(01)00599-8
- Barden N, Reul JM, Holsboer F (1995) Do antidepressants stabilize mood through actions on the hypothalamic-pituitary-adrenocortical system? *Trends Neurosci* 18:6–11. doi:10.1016/0166-2236(95)93942-Q
- Belke TW, Garland T Jr (2007) A brief opportunity to run does not function as a reinforcer for mice selectively bred for high daily wheel-running rates. *J Exp Anal Behav* 88:199–213. doi:10.1901/jeab.2007.62-06
- Board F, Persky H, Hamburg DA (1956) Psychological stress and endocrine functions. *Psychosom Med* 18:324–333
- Boyle MP, Brewer JA, Funatsu M, Wozniak DF, Tsien JZ, Izumi Y, Muglia LJ (2005) Acquired deficit of forebrain glucocorticoid receptor produces depression-like changes in adrenal axis regulation and behavior. *Proc Natl Acad Sci USA* 102:473–478. doi:10.1073/pnas.0406458102
- Brené S, Björnebeck A, Åberg E, Mathé A, Olson L, Werme M (2007) Running is rewarding and antidepressive. *Physiol Behav* 92:136–140. doi:10.1016/j.physbeh.2007.05.015
- Brown GW, Bifulco A, Harris TO (1987) Life events, vulnerability and onset of depression. *Br J Psychiatry* 150:30–42. doi:10.1192/bjp.150.1.30
- Brown ES, Khan DA, Nejtck VA (1999) The psychiatric side effects of corticosteroids. *Asthma Immunol* 83:495–503
- Brown ES, Woolston D, Frol A, Bobadilla L, Khan DA, Hanczyc M (2004) Hippocampal volume, spectroscopy, cognition, and mood in patients receiving corticosteroid therapy. *Biol Psychiatry* 55:538–545. doi:10.1016/j.biopsych.2003.09.010
- Dallman MF, Strack AM, Akana SF, Bradbury MJ, Hanson ES, Scribner KA, Smith M (1993) Feast and famine: critical role of glucocorticoids with insulin in daily energy flow. *Front Neuroendocrinol* 14:303–347. doi:10.1006/fme.1993.1010
- de Kloet ER, Vreugdenhil E, Oitzl MS, Joels M (1998) Brain corticosteroid receptor balance in health and disease. *Endocr Rev* 19:269–301. doi:10.1210/er.19.3.269
- Dishman RK, Berthoud H-R, Booth FW, Cotman CW, Edgerton VR, Fleshner MR, Gandeia SC, Gomez-Pinilla F, Greenwood BN, Hillman CH, Kramer AF, Levin BE, Moran TH, Russo-Neustadt AA, Salamone JD, Van Hoomissen JD, Wade CE, York DA,

- Zigmond MJ (2006) Neurobiology of Exercise. *Obesity* 14:345–356. doi:[10.1038/oby.2006.46](https://doi.org/10.1038/oby.2006.46) (Silver Spring, Md.)
- Duman RS, Monteggia LM (2006) A neurotrophic model for stress-related mood disorders. *Biol Psychiatry* 59:1116–1127. doi:[10.1016/j.biopsych.2006.02.013](https://doi.org/10.1016/j.biopsych.2006.02.013)
- Duman CH, Schlesinger L, Russell DS, Duman RS (2008) Voluntary exercise produces antidepressant and anxiolytic behavioral effects in mice. *Brain Res* 1199:148–158
- Dunn AL, Trivedi MH, Kampert JB, Clark CG, Chambliss HO (2005) Exercise treatment for depression: efficacy and dose response. *Am J Prev Med* 28:1–8. doi:[10.1016/j.amepre.2004.09.003](https://doi.org/10.1016/j.amepre.2004.09.003)
- Dunner D, Patrick V, Fieve RR (1979) Life events at the onset of bipolar affective illness. *Am J Psychiatry* 136:508–511
- El Yacoubi M, Bouali S, Popa D, Naudon L, Leroux-Nicollet I, Hamon M, Costentin J, Adrien J, Vaugeois JM (2003) Behavioral, neurochemical, and electrophysiological characterization of a genetic mouse model of depression. *Proc Natl Acad Sci USA*, 100:6227–6232. doi:[10.1073/pnas.1034823100](https://doi.org/10.1073/pnas.1034823100)
- Garland T Jr (2003) Selection experiments: An underutilized tool in biomechanics and organismal biology. In: Bels VL, Gasc J-P, Casinos A (eds) *Vertebrate biomechanics and evolution*. BIOS Scientific Publishers, Oxford, pp 23–56
- Garland T Jr, Rose MR (eds) (2009) *Experimental evolution: concepts, methods, and applications of selection experiments*. University of California Press, Berkeley (in press)
- Garland T Jr, Morgan MT, Swallow JG, Rhodes JS, Girard I, Belter JG, Carter PA (2002) Evolution of a small-muscle polymorphism in lines of house mice selected for high activity levels. *Evol Int J Org Evol* 56:1267–1275
- Girard I, Garland T Jr (2002) Plasma corticosterone response to acute and chronic voluntary exercise in female house mice. *J Appl Physiol* 92:1553–1561
- Gold P, Loriaux DL, Roy A, Kling MA, Calabrese JR, Kellner CH, Nieman LK, Post RM, Pickar D, Gallucci W, Averginos P, Paul S, Oldfield EH, Cutler GB, Chrousos GP (1986) Responses to corticotropin-releasing hormone in the hypercortisolism of depression and Cushing's disease. *N Engl J Med* 314:1329–1335
- Gomes FR, Rezende EL, Malisch JL, Lee SK, Rivas DA, Kelly SA, Lytle C, Yaspelkis BB III, Garland T Jr (2009) Glycogen storage and muscle glucose transporters (GLUT-4) of mice selectively bred for high voluntary wheel running. *J Exp Biol* (in press)
- Greenwood BN, Foley TE, Day HEW, Campisi J, Hammack SH, Campeau S, Maier SF, Fleshner M (2003) Freewheel running prevents learned helplessness/behavioral depression: role of dorsal raphe serotonergic neurons. *J Neurosci* 23:2889–2898
- Greenwood BN, Foley TE, Burhans D, Maier SF, Fleshner M (2005) The consequences of uncontrollable stress are sensitive to duration of prior wheel running. *Brain Res* 1033:164–178. doi:[10.1016/j.brainres.2004.11.037](https://doi.org/10.1016/j.brainres.2004.11.037)
- Gregus A, Wintink AJ, Davis AC, Kalynchuk LE (2005) Effect of repeated corticosterone injections and restraint stress on anxiety and depression-like behavior in male rats. *Behav Brain Res* 156:105–114. doi:[10.1016/j.bbr.2004.05.013](https://doi.org/10.1016/j.bbr.2004.05.013)
- Hammen C, Davila J, Brown GW, Ellicott A, Gitlin M (1992) Psychiatric history and stress: predictors of severity of unipolar depression. *J Abnorm Psychiatry* 101:45–52. doi:[10.1037/0021-843X.101.1.45](https://doi.org/10.1037/0021-843X.101.1.45)
- Henderson ND (1989) Interpreting studies that compare high- and low-selected lines on new characters. *Behav Genet* 19:473–502. doi:[10.1007/BF01066250](https://doi.org/10.1007/BF01066250)
- Henderson ND (1997) Spurious associations in unreplicated selected lines. *Behav Genet* 27:145–154. doi:[10.1023/A:1025689425738](https://doi.org/10.1023/A:1025689425738)
- Holmes P (2003) Rodent models of depression: reexamining validity without anthropomorphic inference. *Crit Rev Neurobiol* 15:143–174. doi:[10.1615/CritRevNeurobiol.v15.i2.30](https://doi.org/10.1615/CritRevNeurobiol.v15.i2.30)
- Holsboer F (2000) The corticosteroid receptor hypothesis of depression. *Neuropsychopharmacology* 23:477–501. doi:[10.1016/S0893-133X\(00\)00159-7](https://doi.org/10.1016/S0893-133X(00)00159-7)
- Johnson RA, Rhodes JS, Jeffrey SL, Garland T Jr, Mitchell GS (2003) Hippocampal brain-derived neurotrophic factor but not neurotrophin-3 increases more in mice selected for increased voluntary wheel running. *Neuroscience* 121:1–7. doi:[10.1016/S0306-4522\(03\)00422-6](https://doi.org/10.1016/S0306-4522(03)00422-6)
- Johnson SA, Fournier NM, Kalynchuk LE (2006) Effect of different doses of corticosterone in depression-like behavior and HPA axis responses to a novel stressor. *Behav Brain Res* 168:280–288. doi:[10.1016/j.bbr.2005.11.019](https://doi.org/10.1016/j.bbr.2005.11.019)
- Kalynchuk LE, Gregus A, Boudreau D, Perrot-Sinal TS (2004) Corticosterone increases depression-like behavior, with some effects on predator odor-induced defensive behavior, in male and female rats. *Behav Neurosci* 118:1365–1377. doi:[10.1037/0735-7044.118.6.1365](https://doi.org/10.1037/0735-7044.118.6.1365)
- Kaneko M, Hoshino Y, Hashimoto S, Okano T, Kumashiro H (1993) Hypothalamic-pituitary-adrenal axis function in children with attention-deficit hyperactivity disorder. *J Autism Dev Disord* 23:59–65. doi:[10.1007/BF01066418](https://doi.org/10.1007/BF01066418)
- Kelly WF, Checkley SA, Bender DA, Mashiter K (1983) Cushing's syndrome and depression- a prospective study of 26 patients. *Br J Psychiatry* 142:16–19. doi:[10.1192/bjp.142.1.16](https://doi.org/10.1192/bjp.142.1.16)
- Malisch JL (2007) Micro-evolutionary change in the hypothalamic-pituitary-adrenal axis in mice selectively bred for high voluntary wheel running. Ph.D. Dissertation, University of California, Riverside
- Malisch JL, Saltzman W, Gomes FR, Rezende EL, Jeske DR, Garland T Jr (2007) Baseline and stress-induced plasma corticosterone concentrations of mice selectively bred for high voluntary wheel running. *Physiol Biochem Zool* 80:146–156. doi:[10.1086/508828](https://doi.org/10.1086/508828)
- Malisch JL, Breuner CW, Gomes FR, Chappell MA, Garland T Jr (2008) Circadian pattern of total and free corticosterone concentrations, corticosteroid-binding globulin, and physical activity in mice selectively bred for high voluntary wheel-running behavior. *Gen Comp Endocrinol* 156:210–217
- Malisch JL, Kelly SA, Bhanvadia A, Blank KM, Marsik RL, Platzer EG, Garland T Jr (2009) Lines of mice with chronically elevated baseline corticosterone are more susceptible to a parasitic nematode infection. *Zoology (Jena, Germany)* (in press)
- Morgan WP (1985) Affective beneficence of vigorous physical activity. *Med Sci Sports Exerc* 17:94–100
- Nehrenberg DL, Hua K, Estrada-Smith D, Garland Jr T, Pomp D (2008) Voluntary exercise and its effects on body composition depend on genetic selection history. *Obesity* (in review)
- Parker KJ, Schatzberg AF, Lyons DM (2003) Neuroendocrine aspects of hypercortisolism in major depression. *Horm Behav* 43:60–66. doi:[10.1016/S0018-506X\(02\)00016-8](https://doi.org/10.1016/S0018-506X(02)00016-8)
- Pecoraro N, Gomez F, Dallman MF (2005) Glucocorticoids dose-dependently remodel energy stores and amplify incentive relativity effects. *Psychoneuroendocrinol* 30:815–825. doi:[10.1016/j.psyneuen.2005.03.010](https://doi.org/10.1016/j.psyneuen.2005.03.010)
- Pecoraro N, Dallman MF, Warne JP, Ginsberg AB, Laugero KD, la Fleur SE, Houshyar H, Gomez F, Bhargava A, Akana SF (2006) From Malthus to motive: how the HPA axis engineers the phenotype, yoking needs to wants. *Prog Neurobiol* 79:247–340. doi:[10.1016/j.pneurobio.2006.07.004](https://doi.org/10.1016/j.pneurobio.2006.07.004)
- Porsolt RD, Bertain A, Jalife M (1977) Behavioral despair in mice: a primary screening test for antidepressants. *Arch Int Pharmacodyn Ther* 229:327–336
- Post F (1962) The significance of affective symptoms in old age, Maudsley monograph 10. Oxford University Press, London
- Ransford CP (1982) A role for amines in the antidepressant effect of exercise: a review. *Med Sci Sports Exerc* 14:1–10. doi:[10.1249/00005768-198214010-00001](https://doi.org/10.1249/00005768-198214010-00001)

- Rhodes JS, Hosack GR, Girard I, Kelly AE, Mitchell GS, Garland T Jr (2001) Differential sensitivity to acute administration of cocaine, GBR 12909, and fluoxetine in mice selected for hyperactive wheel-running behavior. *Psychopharmacology* 158:120–131. doi:[10.1007/s002130100857](https://doi.org/10.1007/s002130100857)
- Rhodes JS, Gammie SC, Garland T Jr (2003) Patterns of brain activity associated with variation in voluntary wheel-running behavior. *Behav Neurosci* 117:1243–1256. doi:[10.1037/0735-7044.117.6.1243](https://doi.org/10.1037/0735-7044.117.6.1243)
- Rhodes JS, Gammie SC, Garland T Jr (2005) Neurobiology of mice selected for high voluntary wheel-running activity. *Integrative Comp Biol* 45:438–455. doi:[10.1093/icb/45.3.438](https://doi.org/10.1093/icb/45.3.438)
- Ridder S, Chourbaji S, Hellweg R, Urani A, Zacher C, Schmid W, Zink M, Hortnagl H, Flor H, Henn FA, Schutz G, Gass P (2005) Mice with genetically altered glucocorticoid receptor expression show altered sensitivity for stress-induced depressive reactions. *J Neurosci* 25:6243–6250. doi:[10.1523/JNEUROSCI.0736-05.2005](https://doi.org/10.1523/JNEUROSCI.0736-05.2005)
- Sapolsky RM, Plotsky PM (1990) Hypercortisolism and its possible neural bases. *Biol Psychiatry* 27:937–952. doi:[10.1016/0006-3223\(90\)90032-W](https://doi.org/10.1016/0006-3223(90)90032-W)
- Sokal RR, Rohlf FJ (1981) *Biometry*, 2nd edn. W. H. Freeman and Co., San Francisco
- Steru L, Chermat R, Thierry B, Simon P (1985) The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology* 85:367–370. doi:[10.1007/BF00428203](https://doi.org/10.1007/BF00428203)
- Stone EA, Lin Y (2008) An anti-immobility effect of exogenous corticosterone in mice. *Eur J Pharmacol* 580:135–142. doi:[10.1016/j.ejphar.2007.10.045](https://doi.org/10.1016/j.ejphar.2007.10.045)
- Strohle A, Holsboer F (2003) Stress responsive neurohormones in depression and anxiety. *Pharmacopsychiatry* 36:S207–S214
- Swallow JG, Carter PA, Garland T Jr (1998) Artificial selection for increased wheel-running behavior in house mice. *Behav Genet* 28:227–237. doi:[10.1023/A:1021479331779](https://doi.org/10.1023/A:1021479331779)
- Swallow JG, Koteja P, Carter PA, Garland T Jr (1999) Artificial selection for increased wheel-running activity in house mice results in decreased body mass at maturity. *J Exp Biol* 202:2513–2520
- Yehuda R, Southwick SM, Nussbaum G, Wahby V, Giller EL, Mason JW (1990) Low urinary cortisol excretion in patients with posttraumatic stress disorder. *J Nerv Ment Dis* 187:366–369. doi:[10.1097/00005053-199006000-00004](https://doi.org/10.1097/00005053-199006000-00004)
- Young EA, Lopez JF, Murphy-Weinberg V, Watson SJ, Akil H (2003) Mineralocorticoid receptor function in major depression. *Arch Gen Psychiatry* 60:24–28
- Zheng H, Liu Y, Li W, Yang B, Chen D, Wang X, Jiang Z, Wang H, Wang Z, Cornelissen G, Halberg F (2006) Beneficial effects of exercise and its molecular mechanisms on depression in rats. *Behav Brain Res* 168:47–55. doi:[10.1016/j.bbr.2005.10.007](https://doi.org/10.1016/j.bbr.2005.10.007)