BRIEF COMMUNICATION

Acute Restraint Stress Alters Wheel-Running Behavior Immediately Following Stress and up to 20 Hours Later in House Mice

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ABSTRACT

In vertebrates, acute stressors—although short in duration—can influence physiology and behavior over a longer time course, which might have important ramifications under natural conditions. In laboratory rats, for example, acute stress has been shown to increase anxiogenic behaviors for days after a stressor. In this study, we quantified voluntary wheel-running behavior for 22 h following a restraint stress and glucocorticoid levels 24 h postrestraint. We utilized mice from four replicate lines that have been selectively bred for high voluntary wheel-running activity (HR mice) for 60 generations and their nonselected control (C) lines to examine potential interactions between exercise propensity and sensitivity to stress. Following 6 d of wheel access on a 12L:12D photo cycle (0700–1900 hours, as during the routine selective breeding protocol), 80 mice were physically restrained for 40 min, beginning at 1400 hours, while another 80 were left undisturbed. Relative to unrestrained mice, wheel running increased for both HR and C mice during the first hour postrestraint ($P < 0.0001$) but did not differ 2 or 3 h postrestraint. Wheel running was also examined at four distinct phases of the photoperiod. Running in the period of 1600–1840 hours was unaffected by restraint stress and did not differ statistically between HR and C mice. During the period of peak wheel running (1920–0140 hours), restrained mice tended to run fewer revolutions (−11%; two-tailed $P = 0.0733$), while HR mice ran 473% more than C ($P = 0.0008$), with no restraint × line type interaction. Wheel running declined for all mice in the latter part of the scotophase (0140–0600 hours), restraint had no statistical effect on wheel running, but HR again ran more than C (+467%; $P = 0.0122$). Finally, during the start of the photophase (0720–1200 hours), restraint increased running by an average of 53% ($P = 0.0443$) in both line types, but HR and C mice did not differ statistically. Mice from HR lines had statistically higher plasma corticosterone concentrations than C mice, with no statistical effect of restraint and no interaction between line type and restraint. Overall, these results indicate that acute stress can affect locomotor activity (or activity patterns) for many hours, with the most prominent effect being an increase in activity during a period of typical inactivity at the start of the photophase, 15–20 h poststressor.

Keywords: behavior, corticosterone, glucocorticoids, locomotion, selection experiment, stress, vigilance, voluntary exercise.

Introduction

Acute stressors are, by definition, short in duration; therefore, the physiological and behavioral effects produced by acute stress are often assumed to be transient. However, several laboratory studies of rodents have reported behavioral changes 24 h after a single acute stress exposure, with a focus largely on anxiety-like behavior (Armario et al. 1990; Calvo and Volosin 2001; for review, see Armario et al. 2008). As one example, previously stressed rats spent less time exploring the open arms of the elevated plus maze (Korte 2001). This result is interpreted as increased anxiety-like behavior and has also been described as increased vigilance. From an evolutionary perspective, these laboratory findings may be reminiscent of a selective advantage conferred to individuals who were more anxious or vigilant for a period of time following a stressful event.

A likely physiological explanation for longer-term (e.g., 24–72 h) changes in behavior following acute stress is elevated glucocorticoid levels and increased glucocorticoid receptor binding, which have also been shown to increase for up to 24 h following acute stress (Fleshner et al. 1995; Deak et al. 1999; Armario et al. 2008; Malisch et al. 2010). An increase in glucocorticoid level,

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a decrease in corticosterone-binding globulin level, and thus a higher unbound or free glucocorticoid level following laboratory stressors—such as immobilization and handling stress—have been well documented (Fleshner et al. 1995; Deak et al. 1999; Armario et al. 2008; Malisch et al. 2010). Furthermore, Deak et al. (1999) demonstrated that restraint stress in rats results in increased occupancy of specific glucocorticoid receptors (mineralocorticoid and glucocorticoid receptors) for 24–72 h. Elevated receptor occupancy indicates that elevated glucocorticoid levels likely affect behavioral and/or physiological traits for up to 72 h.

Given the sustained effects of acute stressors on the physiological stress response, short periods of stress likely have sustained effects on behavior and physical activity in general, but to date, this remains unevaluated. In this study, we examined changes in typical daily activity for laboratory rodents (voluntary wheel-running behavior; see also Meijer and Robbers 2014) in the 22 h following acute (40-min) restraint stress. We utilized a novel study system: mice from lines selectively bred for high voluntary wheel-running behavior (HR) as well as their nonselected control (C) lines (Swallow et al. 1998; Garland 2003; Careau et al. 2013). The HR lines have chronically elevated baseline corticosterone (Girard and Garland 2002; Malisch et al. 2007, 2008; Downs et al. 2012), the primary glucocorticoid in mice, and HR males are more prone to anxiety-like behavior when wheel access is removed, spending more time floating in the forced-swim test as compared with C males (Malisch et al. 2009).

Using these lines, we can assess changes in behavior following stress in C mice as well as mice with a predisposition for increased wheel running, which is of interest because of the presumed anxiolytic effects of exercise in humans (for a review, see Salmon 2001). Interestingly, exercise has been shown to decrease, increase, or have no effect on anxiety-like behaviors in several rodent studies (Greenwood et al. 2003; Binder et al. 2004; Burghardt et al. 2004; Van Hoomissen et al. 2004; Pietropaolo et al. 2006; Duman et al. 2008; Leasure and Jones 2008; Salam et al. 2009; Fuss et al. 2010). Furthermore, mice exposed to a foot shock stressor have been shown to increase wheel running for at least 3 h immediately following the stress procedure. This behavioral response was interpreted as a self-selected method of reducing anxiety (Sibold et al. 2011).

We predicted that acute stress would increase wheel running, as seen by Sibold et al. (2011), for both HR and C mice and that HR mice would run more than C because the former are generally more active and more sensitive to some stressors that may induce anxiety-like behavior (Malisch et al. 2009). We also predicted that the increase in activity would persist for up to 22 h (the length of the experiment), since corticosterone elevation is expected to be increased for at least 24 h following restraint, on the basis of studies of rats and Japanese quail (Fleshner et al. 1995; Deak et al. 1999; Armario et al. 2008; Malisch et al. 2010). This line of research is interesting because it may provide additional clarity for rodent exercise models of anxiety/depression and because a finding of a change in circadian locomotor activity would aid in making predictions about the ecological or evolutionary significance of changes in behavior that result from acute stress in free-living populations.

Methods

Experimental Animals

Subjects were adult (7–9 wk old) male mice (*Mus domesticus*) from the sixtieth generation of an ongoing artificial selection experiment (Swallow et al. 1998). In this experiment, four replicate HR lines are bred for the number of revolutions run on days 5 and 6 of a 6-d period of wheel access when they are young adults. An additional four lines are tested for wheel running but then bred without regard to their running. These lines serve as controls for random genetic processes (e.g., drift). In all eight lines, mice are weaned at 21 d of age, toe clipped for identification, and housed randomly in same-sex groups of four until the time of wheel testing. The light cycle is maintained at 12L:12D (0700–1900 hours), and food and water are given ad lib. All procedures in the selection experiment and for this study were approved by the Institutional Animal Care and Use Committee of the University of California, Riverside, an Association for Assessment and Accreditation of Laboratory Animal Care International–accredited institution.

Wheel Running

Two male mice from each of 10 families per line were used in this study ($N = 160$). This experiment was conducted during the routine selection procedure (Swallow et al. 1998), with the addition of a seventh day of wheel access. Following the typical selection protocol, wheel testing (and therefore this experiment) took place in three batches over three consecutive weeks, with one-third of the mice being tested each week. Following 6 d of wheel access, 80 mice (half HR and half C lines) were removed from their cage, subjected to a 40-min restraint stressor (Malisch et al. 2007; see "Acute Restraint Stressor"), and returned to their home cage with wheel access. Wheel-running activity was monitored for all mice for an additional 22 h (day 7). All mice were weighed after being removed from wheels on day 7.

Acute Restraint Stressor

Restraint tubes were constructed of clear acrylic tubes (internal diameter 26 mm, length 150 mm) with 6-mm-diameter ventilation holes (Malisch et al. 2007). A two-holed rubber stopper sealed one end, and one-quarter-inch hardware cloth prevented escape from the other end. The mice were able to move horizontally but unable to reverse within the tube. To minimize sensory stimulation, the tubes were placed in padded opaque housing cages. Total restraint duration was 40 min, which has been previously shown to significantly elevate plasma corticosterone approximately twofold in both HR and C lines (Malisch et al. 2007). Restraint periods occurred between 1430 and 1600 hours.

Blood Sampling

As previously described by Downs et al. (2012), blood samples were taken from half of the mice in this study ($N = 80$; 20 HR restrained, 20 HR not restrained, 20 C restrained, and 20 C not

restrained) ~24 h postrestraint (between 1430 and 1600 hours). Blood was acquired from the retro-orbital sinus (Hoff 2000) under isoflurane sedation (Malisch et al. 2009). Precautions were taken to minimize stress to the mice before and during the bleeding process, and blood was acquired within 3 min of removal from the cage. Blood samples were centrifuged (13,300 rpm) at 4°C, then plasma was decanted and maintained at −80°C until assays. Total plasma corticosterone concentration was determined in duplicate with a commercially available enzyme immunoassay kit from Assay Designs (Ann Arbor, MI), following previous protocols (Malisch et al. 2008).

Statistical Analyses

The effects of selection history (line type: HR vs. C) and of physical restraint on wheel running on day 7 were assessed with a two-way mixed model ANCOVA, where selection history and restraint were fixed effects and line was a random effect nested within line type (SAS Procedure Mixed). The statistical models allowed for different estimates of variance for the HR and C lines (see Garland et al. 2011). Age and a measure of wheel freeness (Copes et al. 2015) were used as covariates in all analyses of wheel running. On the basis of examination of the circadian pattern of wheel-running activity found in previous studies with these lines of mice (Girard and Garland 2002; Malisch et al. 2008; Malisch et al. 2009) and also results from this study, wheel revolutions were summed for each of four behaviorally distinct periods: 1600–1840 hours, when lights are on and mice are typically inactive; 1920–0140 hours, when lights are off and during the period of peak wheel running; 0140–0600 hours, when lights are off but wheel running declines rapidly; and 0720–1200 hours, when lights are on and mice exhibit little wheel running. We expected that the difference between HR and C mice as well as possible effects of restraint would vary among these time periods, although we did not have more specific predictions. In addition, following Sibold et al. (2011), we examined the first 3 h immediately following restraint for each individual, beginning from the time that each mouse was returned to its wheel. Because half of the mice were not restrained, we assigned individuals in adjacent cages as having the same start time postrestraint for purposes of these comparisons. A priori, we considered differences among groups as statistically significant if $P < 0.05$.

Results and Discussion

During the first hour immediately following restraint (fig. 1), restrained animals ran significantly more than did nonrestrained individuals ($P < 0.0001$), with no statistical effect of line type ($P = 0.5586$) or an interaction ($P = 0.1594$), on the basis of analyses of log-transformed data. During the second and third hour, neither of the main effects nor the interaction term was statistically significant (all $P > 0.43$). Given the anxiolytic effects of exercise, other researchers have hypothesized that wheel running would increase following acute stress in mice familiar with activity wheels (Sibold et al. 2011). As hypothesized, more than 3 h following acute stress, Sibold et al. (2011) found a significant

Figure 1. Wheel running during the first 3 h following restraint. During the first hour, restrained mice ran significantly more than did nonrestrained ones ($P < 0.0001$). Statistical tests were performed on log-transformed data (see text for other significance levels), but for simplicity, figure depicts least squares means and standard errors from analyses of raw data with SAS Procedure Mixed. One revolution = 1.12 m.

increase in wheel-running distance in the acute stress (foot shock) group as compared with mice that were handled but not shocked. Our results support the findings of Sibold et al. (2011), with the most dramatic increase in wheel revolutions in the first hour following acute stress by foot shock (Sibold et al. 2011) or restraint (this study). We predicted that HR mice would run more than C mice following restraint because HR mice typically are more active and have higher baseline corticosterone. However, the line type effect was not statistically significant, suggesting that the acute anxiogenic effect of restraint stress was similar for HR and C mice. Interestingly, despite having elevated baseline corticosterone, HR mice do not have higher corticosterone levels postrestraint than C mice (Malisch et al. 2007). Thus, if anxiogenic behavioral effects of stress are mediated by corticosterone, then a difference between HR and C lines would not be expected.

Analysis of the entire amount of wheel running during the day (22 h) following restraint stress indicated a highly significant effect of line type ($P = 0.0001$) but no effect of restraint ($P = 0.5496$) and no interaction ($P = 0.7449$). However, wheel-running behavior generally follows a distinct circadian pattern in rodents, and these mice are no exception (Girard and Garland 2002; Malisch et al. 2008, 2009; fig. 2). Therefore, we evaluated wheel running during four behaviorally distinct periods: lights on, when mice are typically sleeping; first half of lights out, which encompasses peak wheel running; second half of lights out, when activity rapidly decreases; and lights on again, while mice typically show little activity. Wheel running following restraint and before lights out (the onset of the active period; 1600–1840 hours) was unaffected by restraint stress ($P = 0.6096$) and did not differ statistically between HR and C mice ($P = 0.2178$), with no interaction ($P = 0.8633$; figs. 2, 3*A*; least squares means + SEs = 259 + 40 revolutions for unrestrained C mice, 293 + 41 for restrained C mice, 656 + 316 for unrestrained HR mice, 726 + 315 for restrained HR mice). Because this time period is during a typical period of inactivity for both HR and C mice, the lack of difference between HR and C lines was not unexpected.

During the period of peak wheel running (1920–0140 hours), analysis of all the animals revealed that restrained mice tended to run fewer revolutions (−11%; two-tailed $P = 0.0733$). As expected, HR mice ran 473% more than C ($P = 0.0008$) but with no restraint × line type interaction ($P = 0.4089$; figs. 2, 3*B*; least squares means + SE = 1,787 + 275 revolutions for unrestrained C mice, 1,522 + 276 for restrained C mice, 8,177 + 998 for unrestrained HR mice, 7,467 + 996 for restrained HR mice). Wheel running declined in the latter part of the scotophase (0140–0600 hours; fig. 3*C*), restraint had no statistical effect on wheel running ($P = 0.8046$), but HR continued to run more than C (+467%; $P = 0.0122$), with no interaction ($P = 0.9392$; least squares means + SE = 602 + 118 revolutions for unrestrained C mice, 575 + 119 for restrained C mice, 2,774 + 619 for unrestrained HR mice, 2,722 + 617 for restrained HR mice).

Finally, during the start of the photophase (0720–1200 hours), restraint significantly increased wheel running by an average of 53% ($P = 0.0443$; fig. 3*D*) in both line types, but HR and C mice did not differ statistically ($P = 0.4563$), and we found no restraint × line type interaction ($P = 0.2166$; least squares means + SE = 336 + 94 revolutions for unrestrained C mice, 421 + 95 for restrained C mice, 476 + 345 for unrestrained HR mice, 825 + 343 for restrained HR mice). This finding is unique and particularly interesting because this increase occurred 16–22 h poststressor. An increase in activity during a typical period of little or no activity (lights on) may indicate increased vigilance, with animals being awake and active, as opposed to quiet rest. Our results are also consistent with the finding that 1 h of

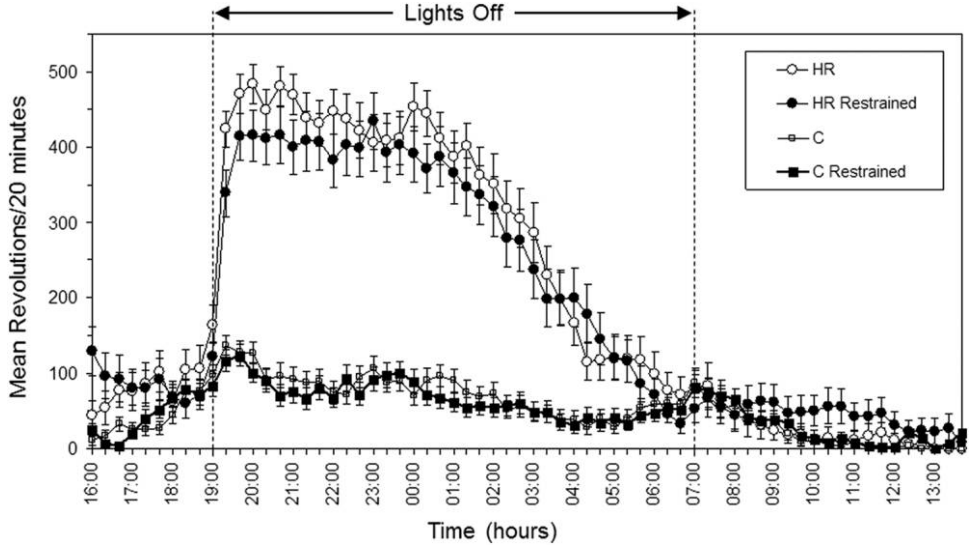

Figure 2. Overall circadian pattern of wheel running on the seventh day of wheel access in high-runner mice (open circles), restrained high-runner mice (filled circles), control mice (open squares), and restrained control mice (filled squares) ± SEM . Mice in the restraint group were restrained for 40 min between 1430 and 1600 hours on day 6 of wheel access. One revolution = 1.12 m.

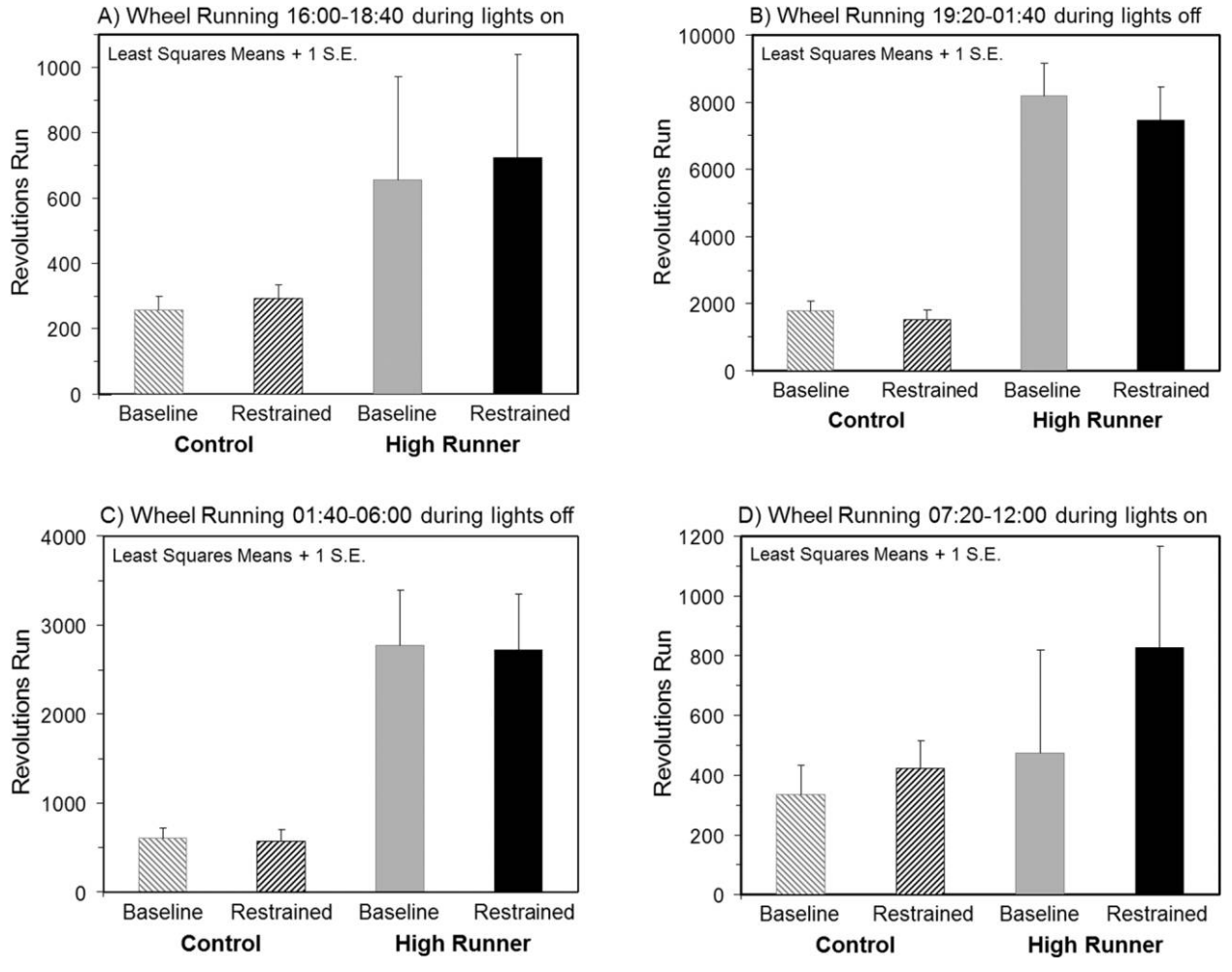

Figure 3. Wheel running during four behaviorally distinct (see fig. 2) parts of the circadian cycle. *A*, 1600–1840 hours, when lights are on and mice are typically inactive. *B*, 1920–0140 hours, when lights are off and the period of peak wheel running occurs. *C*, 0140–0600 hours, when lights are off but wheel running declines rapidly. *D*, 0720–1200 hours, when lights are on and mice exhibit little wheel running. Photophase was 0700–1900 hours. Note the very different *Y*-axis ranges. One revolution = 1.12 m.

restraint leads to sleep-wake alterations in both control rats as well as rats prenatally exposed to restraint stress (Dugovic et al. 1999).

As noted in the introduction, an increase in glucocorticoid levels as well as receptor occupancy are candidates for a physiological explanation for changes in behavior following acute stress. For example, in the elevated plus maze, a test used to indicate anxiety in rodent systems, exploration of the open arms is reduced (suggesting increased anxiety) 24 h after a 15-min restraint stress in rats (Albonetti and Farabollini 1992; Calvo et al. 1998). Restraint stress leads to a robust increase in glucocorticoid levels (reviewed in Paré and Glavin 1986; Glavin et al. 1994; Buynitsk and Mostofsky 2009; see also Malisch et al. 2007). Interestingly, the anxiogenic effects of restraint stress are eliminated when rats are treated with metyrapone 3 h before the restraint. Metyrapone is a 11-β-hydroxylase inhibitor that prevents glucocorticoid synthesis and thus any increase in glucocorticoids following a stressor. Taken together, these studies provide solid evidence that stress-induced changes in behavior are mediated partly by glucocorticoids.

As reported previously (Downs et al. 2012), analyses of plasma samples taken from half of these same individual mice at the end of the seventh day of wheel access indicated that HR lines had statistically higher plasma corticosterone concentrations than C mice (two-tailed $P = 0.0158$), with no statistical effect of restraint (two-tailed $P = 0.1451$) and no interaction between line type and restraint ($P = 0.2742$). Although the effect was not statistically significant (in contrast to other studies; see Fleshner et al. 1995; Deak et al. 1999; Armario et al. 2008; Malisch et al. 2010), restraint did tend to have a positive effect (in the expected direction) on plasma corticosterone concentrations, especially in the HR lines (see fig. 4). Examination of the least squares means for all four experimental groups (fig. 4; not reported in Downs et al. 2012) suggests that HR mice experienced a greater increase in baseline corticosterone the day following restraint stress. This pattern is similar to that shown for wheel running during the start

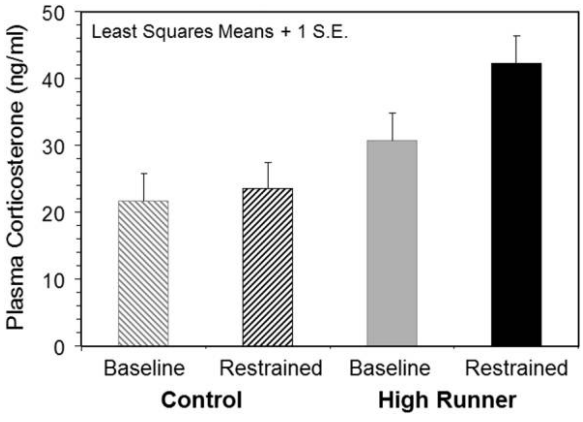

Figure 4. Plasma corticosterone levels ~24 h after half of the mice experienced 40 min of physical restraint (from Downs et al. 2012 but here reported in greater detail). Mice from the selectively bred high-runner lines had significantly higher values than those from control lines ($P = 0.0158$), with no statistical effect of restraint ($P = 0.1451$) and no interaction ($P = 0.2742$).

of the photophase (0720–1200 hours; fig. 3*D*), which is consistent with the hypothesis that elevated circulating corticosterone levels may be one mechanism that can increase activity levels following stress (see introduction; see also fig. 1) but in a genotype-dependent manner. If the effect of acute stress on glucocorticoid level and behavior really is stronger in the HR mice, and given that the HR mice have higher baseline glucocorticoid levels (Girard and Garland 2002; Malisch et al. 2007; Downs et al. 2012), this would represent a pattern consistent with a so-called reactive coping style, characterized as being more vigilant/anxious and having a more active hypothalamic-pituitary-adrenal axis (Koolhaas et al. 1999; see also Crino et al. 2010). From an ecological and evolutionary perspective, changes in circadian locomotor activity—particularly an increase in activity when animals are typically sleeping and a decrease in activity during a typical active period—may represent increased vigilance, a potentially adaptive response if the stressor were an interaction with a predator. This possibility could be tested in tractable wild populations of rodents (e.g., Blumstein et al. 2006).

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