

Risk-assessment and Coping Strategies Segregate with Divergent Intrinsic Aerobic Capacity in Rats

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Metabolic function is integrally related to an individual's susceptibility to, and progression of, disease. Selective breeding for intrinsic treadmill running in rats has produced distinct lines of high- or low-capacity runners (HCR and LCR, respectively) that exhibit numerous physiological differences. To date, the role of intrinsic aerobic capacity on behavior and stress response in these rats has not been addressed and was the focus of these studies. HCR and LCR rats did not differ in their locomotor response to novelty or behavior in the light/dark box. In contrast, immobility in the forced swim test was higher in LCR rats compared with HCR rats, regardless of desipramine treatment. Although both HCR and LCR rats responded to cat odor with decreased exploration and increased risk assessment, HCR rats showed greater contextual conditioning to cat odor. HCR rats exhibited higher expression of corticotropin-releasing hormone in the central nucleus of the amygdala, as well as heavier adrenal and thymus weight. Corticosterone was comparable among HCR and LCR rats at light/dark transitions, and in response to unavoidable cat odor. HCR rats, however, exhibited a greater corticosterone response following the light/dark box. These experiments show that the LCR phenotype associates with decreased risk assessment in response to salient danger signals and passive coping. In contrast, HCR rats show a more naturalistic strategy in that they employ active coping and a more vigilant and cautious response to environmental novelty and salient danger signals. Within this context, we propose that intrinsic aerobic capacity is a central feature mechanistically linking complex metabolic disease and behavior.

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INTRODUCTION

The second half of the twentieth century has witnessed an unfortunate rise in the rates of depression and metabolically related diseases (obesity, diabetes, and cardiovascular disease) in many developing nations (Kopelman, 2000). Concurrently, the prevalence of mental health disorders has continued to rise (Weissman *et al*, 1993) comprising one of the most expensive economic, social, and emotional burdens for industrialized nations, including the United States. Although there is evidence for an association between obesity, cardiovascular disease, diabetes, and metabolic syndrome (MetS) with depression (McIntyre *et al*, 2007), the biological link among these diseases has been tenuous to date. Recent epidemiological work shows a reciprocal relationship between MetS and depression (Bonnet *et al*, 2005; Koponen *et al*, 2008; McIntyre *et al*, 2007; Raikkonen *et al*, 2007), suggesting a biological

commonality shared by metabolic and emotional fitness. It has also been repeatedly documented that stress has the capacity to precipitate and exacerbate poor health and disease, and that disruptions in the stress system are often found in disease states (McEwen and Stellar, 1993). Intriguingly, a great deal of individual variability exists for coping strategies in response to stress (McEwen, 2000). Therefore, the precipitation and progression of disease may be mediated by an individual's ability to employ their optimal coping strategy in response to stress. Although exercise capacity and stress system dysfunction both associate with disease, it has not been determined whether coping strategies are related to differences in intrinsic fitness.

When considering the interactions among metabolism and stress, it is notable that many of the neurochemical systems that regulate metabolism also influence mood and stress. In turn, neuroendocrine systems implicated in mood and stress responsivity also influence metabolism (Kask *et al*, 2001; Koylu *et al*, 2006). Consequently, alterations in neuroendocrine systems by one means (eg, lowered physical activity, increased caloric intake, individual differences in aerobic capacity, etc) may produce changes in other behavioral end points (eg, risk assessment, anxiety, depression, etc), and *vice versa*.

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In 1996, development of contrasting rat strains that diverge for intrinsic aerobic capacity was initiated. Starting from a large founder population, selection for low-capacity runner (LCR) and high-capacity runner (HCR) rats was based on distance run to exhaustion on a motorized treadmill. Currently, at generation 26 of selections, the LCR rats manifest hallmarks of MetS, including elevated triglyceride levels, hypertension, insulin resistance, and visceral adiposity (Gonzalez *et al*, 2006; Hussain *et al*, 2001; Lujan *et al*, 2006; Wisloff *et al*, 2005). Intriguingly, LCR rats are also susceptible to diet-induced obesity, whereas HCR rats are resistant (Noland *et al*, 2007). Therefore, breeding for intrinsic aerobic capacity has produced strains of rats with relatively low and high fitness and disease susceptibility in LCR and HCR animals, respectively.

Given the comorbidity and reciprocal relationship among metabolic disorders, depression, and stress, identifying an animal model that exhibits components of metabolic dysfunction in combination with mood disorders would be an important step in unraveling shared etiology of these diseases. To this end, we sought to investigate the neurobehavioral phenotype and stress system response in the contrasting LCR–HCR rat model, a rodent model of divergent intrinsic aerobic capacity.

In Experiment 1, behavioral and neuroendocrine responses to a novel environment were assessed using the locomotor response to novelty and light/dark box (LDB) paradigms. In Experiment 2, immobility in the forced swim test (FST) was assessed to determine basal levels of ‘depression-like’ behavior and response to antidepressant treatment. In Experiment 3, patterns of behavioral response to cat odor and to the environment where cat odor was encountered were determined. In Experiment 4, we evaluated gene expression in brain regions involved in emotionality and stress response under basal conditions.

METHODS

Rats from the N:NIH stock were selectively bred based on their intrinsic treadmill running capacity to produce two distinct lines with divergent running capacity, the HCR and LCR, respectively (Koch and Britton, 2001). A total of 68 HCR and 68 LCR male rats from the seventeenth generation of selection were used for all experiments described. HCR and LCR rats were screened for intrinsic treadmill running capacity at 10 weeks of age as described in detail elsewhere (Koch and Britton, 2001).

Pairs of HCR or LCR rats were housed in an environmentally controlled vivarium on a 12:12 light/dark cycle with lights on at 0700 hours, with chow and water available *ad libitum*. Animals were housed in an Association for Assessment and Accreditation of Laboratory Animal Care-approved animal facility and all procedures were approved by the University Committee on Use and Care of Animals at The University of Michigan.

Behavioral Testing and Neuroendocrine Assessment

All behavioral testing occurred between 0830 and 1200 hours using separate groups of rats for each experiment starting at 18 weeks of age.

Experiment 1: Response to novelty. Eleven HCR and 11 LCR rats were tested for their locomotor response to novelty and behavior in the LDB as described previously (Kabbaj *et al*, 2000). To determine locomotor response to novelty, rearing and horizontal activity were measured in 5-min bins for 1 h using the locomotor testing rig and computer software created in-house at The University of Michigan. Total locomotion was determined by summing the rearing and horizontal scores.

Testing in the LDB occurred 1 week following assessment of locomotor response to novelty. On the basis of pilot studies, animals were placed in the light chamber and allowed to freely explore the LDB for 5 min. Interruption of the photocells was recorded by a microprocessor and used to determine activity and time spent in each chamber, and the latency for the first transition between chambers.

Blood samples were collected to measure levels of corticosterone following exposure to the light/dark box. Rats were lightly restrained with a towel, and approximately 40 μ l of blood from the tail nick was collected in microcapillary tubes, transferred to a chilled eppendorf tube containing 20 mg/ml EDTA, and subsequently stored on ice until it was spun for 10 min at 3000 r.p.m. at 4°C. Blood samples were collected 24 h before the start of the test, and then at 15, 30, and 90 min after the start of the LDB test.

Following testing in the LDB, rats were handled three times a week during a 2-week washout period. Following the washout period, half the animals were killed in the morning just after lights on, whereas the other half were killed in the evening just before lights off to examine corticosterone levels at the nadir and peak of the circadian cycle, respectively. Adrenal and thymus glands were collected and weighed.

Plasma corticosterone levels were determined using a commercially available radioimmunoassay kit for rat corticosterone (DPC, Los Angeles, CA), as per the manufacturer’s instructions.

Experiment 2: FST with antidepressant treatment. The FST was conducted in a clear Plexiglass cylinder that was 40 cm tall and 18 cm in diameter. The tank was filled with 25°C water to a level that prevented the rat from touching the bottom of the tank with his hind paws while keeping his head above the surface of the water. Testing occurred over 2 consecutive days with a 15-min swim on day 1 and a 5-min swim on day 2. Desipramine (20 mg/kg, intraperitoneally, dissolved in physiological saline) or saline ($n = 7$ /line/drug combination) was injected at 23, 5, and 1 h before the 5-min swim on day 2 as described previously (Detke, 1995). Time spent immobile (defined as the lack of any paw or tail movement) was scored by a trained individual who was blind to the treatment and breeding groups. Data from these experiments are expressed as percent time spent immobile, during 5-min bins.

Experiment 3: Response to cat odor. All experiments using exposure to cat odor were conducted in a black Plexiglas arena (60 cm long \times 26 cm wide \times 36 cm high) under red-light conditions, similar to those described previously (Dielenberg and McGregor, 1999). One 60-cm wall was made of clear Plexiglas along the whole length of the arena, allowing each animal’s behavior in the open arena and hide

box to be observed. One-third of the box was separated by a wall with an open door (6 cm × 6 cm), which constituted the 'hide box'. Animals were habituated to the odor-exposure arena for 20 min a day for 3 days. On all days, animals were placed in the arena facing away from the hide box.

On the 4th day, risk-assessment and defensive behaviors were measured when animals were returned to the arena for 20 min with a 1-inch piece of cat collar containing either: (a) unworn collar that was washed (control), (b) a piece of collar worn by a cat for 3 weeks, or (c) a piece of collar onto which a novel odor provided by 10 µl of strawberry flavoring (McCormick Strawberry Food Flavoring) was placed (Masini *et al*, 2005). Thirty HCR and 30 LCR rats were randomly assigned to one of the three odor groups ($n = 10$ for each line/odor combination). Before placing the rat in the testing arena, the collar containing the odor was placed in the arena at the wall opposite from the hide box as described previously (Dielenberg and McGregor, 1999). To test for contextual conditioning, animals were returned to the testing arena 24-h following exposure to odor (on day 5) for 10 min.

Percent of total time engaged in the following behaviors were determined ((time engaged in behavior/test duration) × 100%): (a) head out; (b) exploration in the open area (outside of hide box); and (c) contact with the odor source. Rearing was included as part of exploration as it was highly correlated with exploration in the open area in pilot studies. The following pre-planned comparisons were considered for *post hoc* analysis: (a) control odor vs cat odor within line; (b) novel odor vs control odor within line; (c) HCR-control vs LCR-control; and (d) HCR-cat vs LCR-cat.

Corticosterone Response to Unavoidable Cat Odor

Corticosterone response to unavoidable psychological stress was determined by placing HCR and LCR animals ($n = 6$ /line) in close proximity to cat odor while confined to the hide box. Animals were habituated to the hide box for 20-min/day for 3 consecutive days. On the 4th day, rats were exposed to cat collar for 20 min. Immediately following the 20-min exposure, rats were euthanized, and tissue and blood samples were collected.

Experiment 4: Basal mRNA expression in limbic and hypothalamic brain regions. Brains from cage-control (untested) HCR ($n = 7$) and LCR ($n = 7$) rats were used for *in situ* hybridization to determine mRNA levels of glucocorticoid receptor (GR; M14053) and mineralocorticoid receptor (MR; M36074) in the hippocampus. In addition, we assessed mRNA levels of corticotrophin-releasing factor (M54987) in the paraventricular hypothalamus and central nucleus of the amygdala. Animals were euthanized by decapitation, brains were removed, frozen in 2-methylbutane chilled to -30°C , and stored at -80°C until further processing.

Brains were sectioned at 10 µm on a Leica cryostat at -16°C , thaw mounted onto poly-L-lysine-coated slides, and stored at -80°C until hybridization. Slides were processed as described previously (Kabbaj *et al*, 2000). Briefly, sections were fixed in 4% paraformaldehyde for 60 min at room temperature. Sections were rinsed in buffer, dehydrated in ethanol, and air-dried before hybridization.

Slides were hybridized overnight at 55°C with ^{35}S -labeled antisense probes for the transcripts listed above. Following hybridization, slides were treated with RNase, rinsed with buffers of decreasing salt concentration, dehydrated in ethanol, and air-dried before being exposed to Kodak Biomax MS Film (Rochester, NY). The resulting autoradiograms were analyzed using Scion Image (Frederick, MD).

Data Analysis

The independent levels of line (HCR and LCR) were applied to the analysis for all dependent variables. Two-tailed *t*-tests were used to compare group differences between HCR and LCR rats for: LDB behavior, treadmill running distance, total locomotion scores, corticosterone response to unavoidable cat odor, thymus weight, and body weight. Adrenal gland weight, corticosterone levels at the transitions between light and dark, the effects of desipramine on immobility during day 2 of the FST, and behavioral responses to cat odor were analyzed by two-way analysis of variance. Two-way analysis of variance with time treated as a repeated measure was used to analyze the corticosterone response to the LDB, locomotor activity in response to novelty, and immobility on day 1 of the FST. Tukey's *post hoc* correction was used to determine differences among groups when significant main effects or interactions were evident. Statistical significance was set at $p < 0.05$ for all analyses.

RESULTS

Experiment 1: Behavioral Responding to Novelty

Treadmill performance and body weight. As expected, selection for intrinsic treadmill capacity resulted in a run time to exhaustion that was significantly shorter in LCR (21.5 ± 0.6 min) compared with HCR (58.7 ± 1.2 min) rats ($t_{20} = 26.9$, $p < 0.0001$; Figure 1a). The distance covered before exhaustion was also significantly less in LCR (320 ± 13 m) compared with HCR (1420 ± 48 m) rats ($t_{20} = 22.4$, $p < 0.0001$; Figure 1b). LCR rats weighed significantly more than HCR rats ($t_{20} = 22.4$, $p < 0.0001$; Figure 1c).

Locomotor activity. Although selective breeding resulted in a large difference in intrinsic treadmill capacity, locomotor activity did not differ between HCR and LCR rats during a 60-min exposure to a novel environment. During this time, both HCR and LCR rats showed similar patterns of locomotor activity (Figure 2). Combined locomotor activity decreased over time in a similar manner for HCR and LCR animals, as indicated by a main effect of time ($F_{1,10} = 29.18$, $p < 0.0001$) as did rearing ($F_{1,10} = 29.18$, $p < 0.0001$) and horizontal activity ($F_{1,10} = 21.60$, $p < 0.0001$). There was no significant effect of line and no significant interaction for combined activity, rearing, or horizontal activity. The cumulative horizontal activity, rearing, and combined number of activity counts for the test did not differ between lines (Figure 2a–c, insets).

Light/dark box. There were no differences in the time spent in the light compartment, or total activity counts in the LDB. There were non-significant trends for LCR rats to have

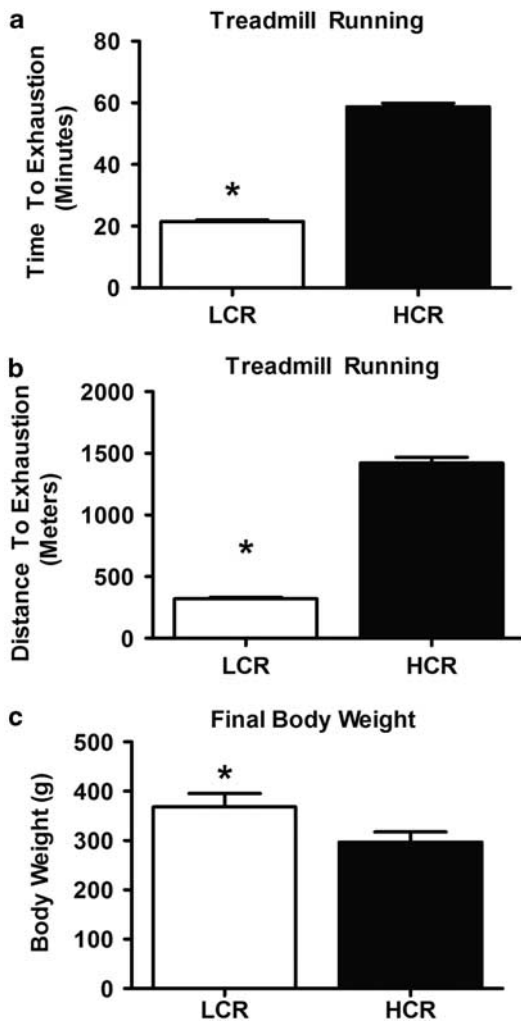


Figure 1 Treadmill performance and body weight. Best time (a) and distance (b) to exhaustion during the 5-day screen for intrinsic aerobic capacity. Final body weight (c) following behavioral testing in Experiment 1. Data are represented as mean \pm SEM; *indicates $p < 0.05$.

a lower latency to transition out of the light compartment ($p = 0.0529$; Figure 3a) and higher activity levels ($p = 0.1245$; Figure 3b) in the light compartment compared with HCR rats.

Corticosterone response to the LDB. Under baseline conditions, HCR and LCR rats had similar levels of corticosterone. At 15 min following the start of the LDB, HCR had significantly higher corticosterone. HCR also had higher corticosterone levels compared with LCR rats at the peak of the corticosterone response (30 min) in both lines. Corticosterone returned to baseline levels 90 min following the start of the LDB (Figure 3c).

Adrenal and thymus weight. A main effect of line was determined for adrenal gland weight, with LCR rats having smaller adrenals compared with HCR rats ($F_{1,34} = 69.58$, $p < 0.0001$; Figure 4a). The left adrenal weighed more than the right adrenal gland, regardless of line ($F_{1,34} = 18.56$, $p = 0.0001$). Thymus gland weight was also lower in LCR rats compared with HCR ($t_{20} = 3.696$, $p = 0.0018$; Figure 4b).

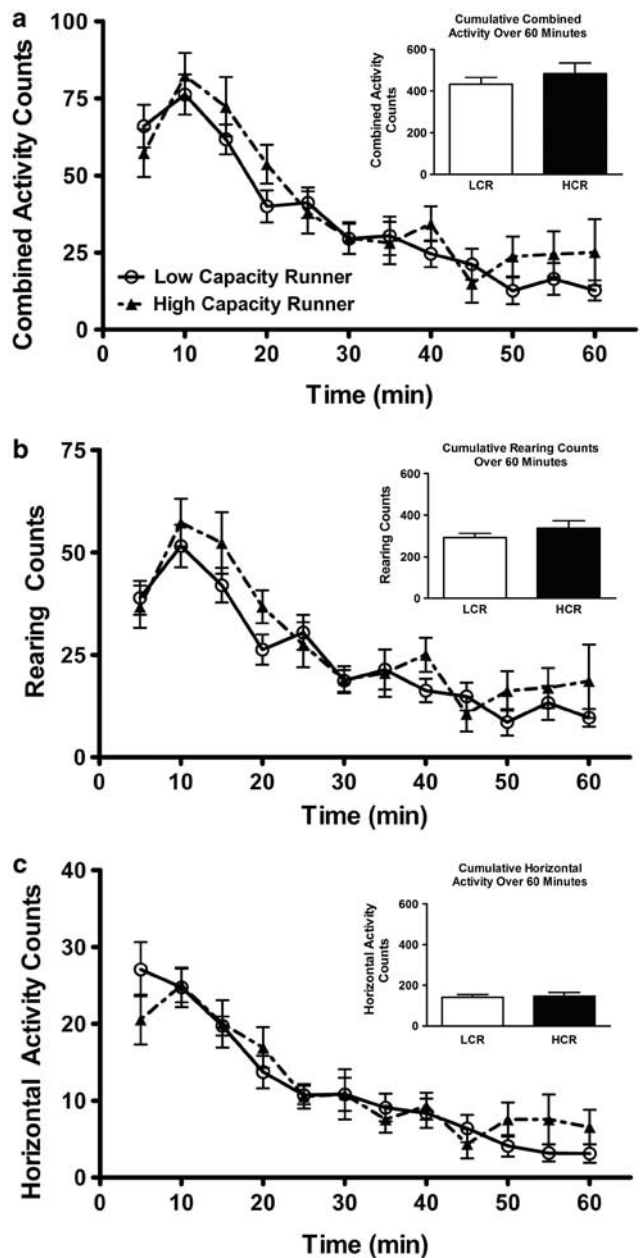


Figure 2 Locomotor response to a novel environment over 60 min. Combined activity (a), rearing (b), and horizontal (c) activity counts for each 5-min bin are represented. Markers and error bars represent the mean \pm standard error of mean, respectively. The inset graphs in each panel represent the cumulative number of counts for each measure summed over the 60-min test.

Basal corticosterone. During tissue collection, trunk blood was collected for the analysis of basal circulating corticosterone levels. No differences were found between HCR and LCR rats for plasma corticosterone levels ($F_{1,20} = 0.08$, $p > 0.05$), regardless of when samples were taken (Figure 4c). A significant main effect of time was apparent with elevated corticosterone levels before lights shutting off compared with corticosterone levels just after the lights turned on ($F_{1,20} = 20.11$, $p = 0.0002$).

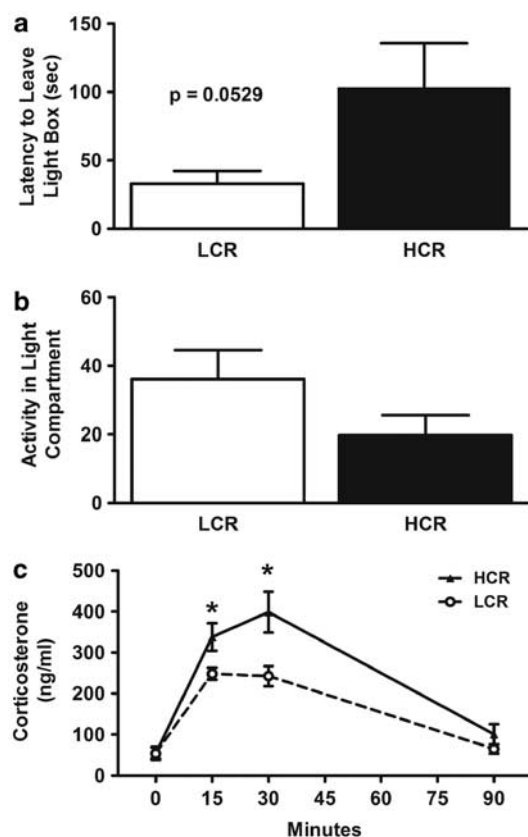


Figure 3 Behavioral (a and b) and corticosterone (c) responses to the light/dark box. Data are represented as mean \pm SEM, *indicates $p < 0.05$.

Experiment 2: FST

During the 15-min swim on day 1, both HCR and LCR rats showed similar increases in immobility as indicated by a significant main effect of time ($F_{1,23} = 447.20$, $p < 0.0001$) and a non-significant time \times line (HCR vs LCR) interaction ($p > 0.09$). No significant main effect of line was found during day 1 (Figure 5a).

The effect of treatment with the antidepressant desipramine on immobility during day 2 of the FST was assessed (Figure 5a and b). Main effects of line ($F_{1,24} = 10.11$, $p = 0.0043$) and drug ($F_{1,24} = 15.47$, $p = 0.0007$) were found for percent immobility during day 2 of the FST, and there was no significant interaction. HCR-desipramine and LCR-desipramine rats showed lower immobility compared with HCR vehicle- and LCR vehicle-treated rats, respectively. Further, HCR vehicle-treated rats showed lower levels of immobility compared with LCR vehicle-treated rats.

Experiment 3: Behavioral Response to Cat Odor

Exposure—20 min odor exposure. Increases in defensive responding and risk assessment are typically displayed by rodents in response to salient ethological cues related to predators (Blanchard *et al*, 2005). A significant main effect of line was found for risk-assessment behavior (ie, time in head out position) ($F_{1,55} = 17.43$, $p = 0.0001$). In addition, there was a significant main effect of odor ($F_{2,54} = 37.90$,

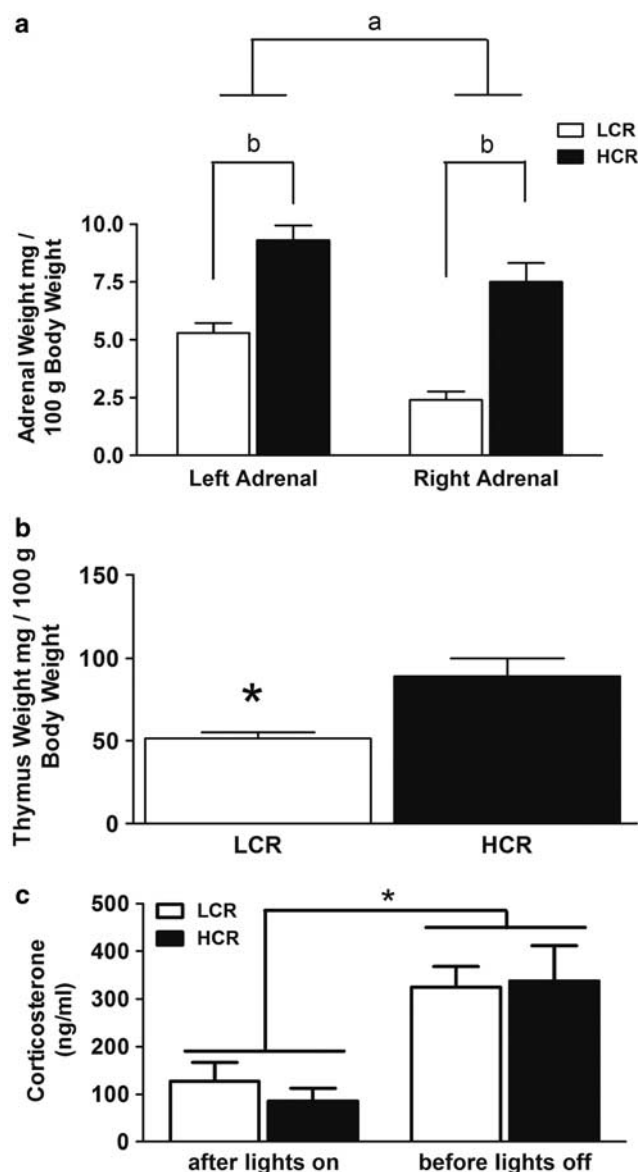


Figure 4 Adrenal (a) and thymus (b) weight per 100 g body weight. Circulating corticosterone at the transition from dark-to-light and light-to-dark (c). For adrenal gland weight ^aindicates a significant difference between left and right adrenal weight ($p < 0.05$), ^bindicates a significant difference between LCR and HCR for adrenal weight ($p < 0.05$). Data are represented as mean \pm SEM, *indicates $p < 0.05$.

$p < 0.0001$) on time spent in risk-assessment behavior. No significant interaction was found between line (HCR/LCR) and odor (Figure 6a, Table 1). HCR-cat and LCR-cat odor-exposed rats both displayed lower exploration compared with control rats of the same line.

Significant main effects of line ($F_{1,55} = 21.64$, $p < 0.0001$) and odor ($F_{2,54} = 29.38$, $p < 0.0001$) were found for time spent in exploration outside of the hide box (Figure 6b, Table 1), whereas no significant interaction was found. *Post hoc* testing indicated that HCR-cat and LCR-cat spent less time exploring outside the hide box compared with HCR-control and LCR-control, respectively. Cat odor-exposed HCR rats also spent less time exploring the open area compared with cat odor-exposed LCR rats. HCR-control

rats spent less time exploring the open area compared with LCR-control rats.

A main effect of line ($F_{1,55} = 5.13$, $p = 0.0277$) was found for the amount of time spent in contact with the odor source (collar), with HCR rats spending less time biting or grabbing the collar compared with LCR rats (Table 1).

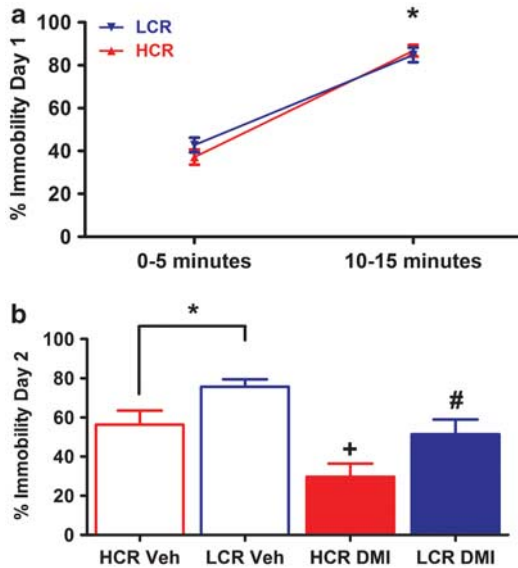


Figure 5 Immobility in the forced swim test. Immobility during the 15-min swim on day 1 in high- or low-capacity runner (HCR and LCR) rats during the 0–5 min bin compared with the 10–15 min bin (a). *A significant increase in immobility over time. Effects of treatment with the antidepressant desipramine (DMI) on immobility during the 5-min swim on day 2 (b). +HCR-DMI different from all other groups. #LCR-DMI different from LCR-vehicle and HCR-DMI. *A significant difference between vehicle-treated HCR and LCR rats. Data are represented as mean \pm SEM.

There was no significant main effect of odor, no interaction, and no significant *post hoc* pair-wise comparisons for time spent in contact with the odor source.

Test—10 min re-exposure to context. At 24 h following the 20-min exposure to the odor, animals were returned to the context in the absence of the odor source and holding clip. Behavioral responding to the context 24-h following exposure to predator odor has been used as a measure of contextual learning (Blanchard *et al*, 2003; Dielenberg and McGregor, 1999).

A significant main effect of line was found for time in head out behavior (risk assessment) ($F_{1,55} = 24.46$, $p < 0.0001$) when rats were returned to the context 24 h after odor exposure (Figure 6c, Table 1). In addition, there was a significant main effect of odor ($F_{2,54} = 11.93$, $p < 0.0001$) on time spent in head out behavior. There was no significant interaction between line (HCR/LCR) and odor.

Significant main effects of line ($F_{1,55} = 26.12$, $p < 0.0001$) and odor ($F_{2,54} = 9.65$, $p < 0.0001$) were found for the amount of time spent exploring outside of the hide box, and no significant interaction was found (Figure 6d, Table 1). *Post hoc* analysis indicated that HCR rats previously exposed to cat odor engaged in less open area exploration compared with HCR-control and LCR-cat odor-exposed rats (Figure 6d). Control odor-exposed HCR rats spent less time exploring the open area compared with control odor-exposed LCR rats. No other significant pair-wise comparisons were found.

Corticosterone response to unavoidable cat odor. Similar levels of corticosterone were found ($p = 0.7473$) in HCR (464 ± 76 ng/ml) and LCR (426 ± 81 ng/ml) rats following 20 min of confinement with a collar worn by a cat.

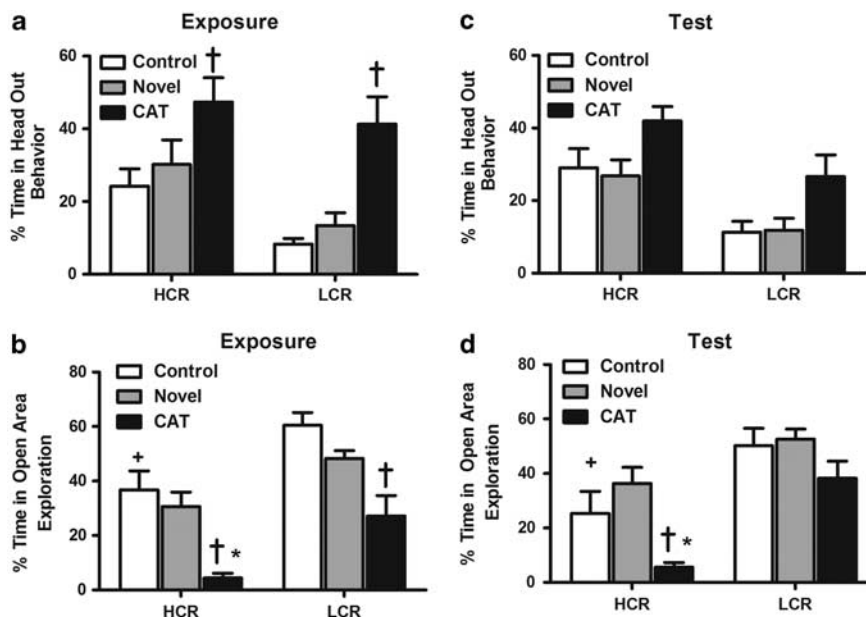


Figure 6 Behavioral responses of high- or low-capacity runner (HCR and LCR) rats to control, cat, or novel (strawberry) odor during odor exposure (a and b) or upon return to the testing arena 24-h later in the absence of an odor source (c and d). *A significant difference between control and cat odor within the same line of rats. #A significant difference among HCR-cat and LCR-cat odor-exposed rats. +A significant difference among HCR-control and LCR-control odor-exposed rats. Data are represented as mean \pm SEM.

Table 1 Behavioral Responses of HCR–LCR Rats in Response to Control, Cat, or Strawberry (novel) Odor

	HCR			LCR			Comparisons between lines	
	Control (mean ± SEM)	Cat (mean ± SEM)	Strawberry (mean ± SEM)	Control (mean ± SEM)	Cat (mean ± SEM)	Strawberry (mean ± SEM)	HCR-cat vs LCR-cat	HCR- cont vs LCR-cont
<i>Odor exposure</i>								
Odor source contact	2.5 ± 0.7	0.7 ± 0.2	1.6 ± 0.4	4.2 ± 1.5	3.1 ± 0.8	3.5 ± 0.7		
Open area exploration	36.8 ± 6.9	4.5 ± 1.7 ^a	30.7 ± 5.3 ^a	60.5 ± 4.5	27.2 ± 7.5 ^a	48.3 ± 3.1 ^a	^b	^c
Head out	24.2 ± 4.8	47.4 ± 6.7 ^a	30.2 ± 6.7 ^a	8.3 ± 1.4	41.3 ± 7.5 ^a	13.4 ± 3.7 ^a		
<i>Test (return to arena)</i>								
Open area exploration	25.3 ± 8.2	5.7 ± 1.6 ^a	36.3 ± 5.9 ^a	50.2 ± 6.1	38.3 ± 6.3	52.6 ± 3.9	^b	^c
Head out	29.0 ± 5.3	41.9 ± 4.0	26.8 ± 4.4	11.3 ± 2.9	26.6 ± 6.0	11.8 ± 3.5		

Results are given for the 20-min odor exposure (top) and the 10-min test that occurred 24-h after odor exposure in the absence of any odor. Data are reported as time engaged in behavior as a percentage of total duration of the behavioral assay. Superscript alphabets indicate significantly different pair-wise comparisons of interest.

^aControl odor vs cat odor (within line).

^bHCR-cat vs LCR-cat.

^cHCR-control vs LCR-control.

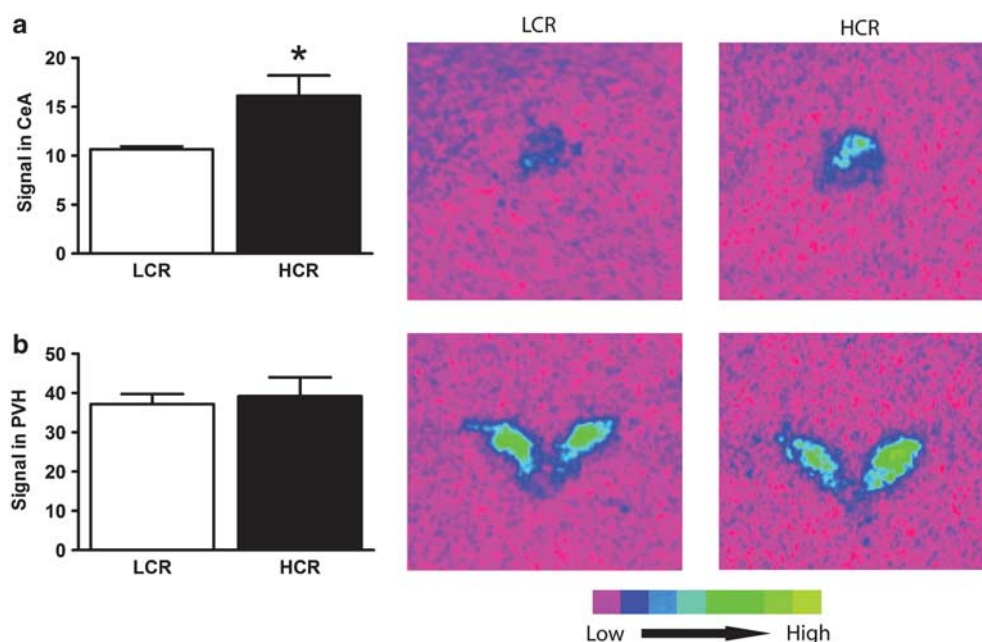


Figure 7 Basal expression of corticotropin-releasing hormone (CRH) mRNA in the central nucleus of the amygdala (CeA; a) and paraventricular hypothalamus (PVH; b). Data are represented as mean ± SEM, *indicates $p < 0.05$.

Experiment 4: Basal mRNA Expression in Limbic and Hypothalamic Brain Regions

Basal expression of corticotropin-releasing hormone (CRH) mRNA was higher in the central nucleus of the amygdala in HCR rats compared with LCR rats ($t_{12} = 2.36$, $p = 0.0424$; Figure 7a). In the paraventricular hypothalamus, basal

expression of CRH mRNA expression did not differ between HCR and LCR animals (Figure 7b).

A significant main effect of hippocampal region (CA1, CA2, CA3, and DG) was found for GR mRNA ($F_{3,40} = 31.98$, $p < 0.0001$; Table 2). There was no significant main effect of line and no significant interaction for line by hippocampal region for GR mRNA expression.

Table 2 Mean Signal Values of Mineralocorticoid and Glucocorticoid Receptor mRNA Expression in Subregions of the Hippocampus

	HCR	LCR
	Mean \pm SEM	Mean \pm SEM
<i>Mineralocorticoid receptor</i>		
CA1	34.7 \pm 2.1	33.1 \pm 2.3
CA2	42.3 \pm 1.8	36.4 \pm 2.0
CA3	23.0 \pm 1.0	23.5 \pm 0.9
DG	28.3 \pm 1.8	29.3 \pm 1.4
<i>Glucocorticoid receptor</i>		
CA1	19.8 \pm 1.2	18.6 \pm 1.2
CA2	5.8 \pm 0.2	5.8 \pm 0.3
CA3	6.2 \pm 0.3	7.0 \pm 0.4
DG	22.9 \pm 0.9	21.2 \pm 1.3

Values are mean \pm SEM.

A significant main effect of hippocampal region (CA1, CA2, CA3, and DG) was found for MR mRNA ($F_{3,40} = 195.7$, $p < 0.0001$; Table 2). There was no significant main effect of line and no significant interaction for line by hippocampal region for MR mRNA expression.

DISCUSSION

These experiments show that (1) animals with divergent intrinsic aerobic capacity display relatively similar patterns of behavior in response to environmental novelty under basal conditions; (2) LCR rats show higher immobility in the FST compared with HCR rats following vehicle or antidepressant treatment; (3) HCR rats show greater risk assessment, decreased exploration, and a sustained behavioral response suggestive of contextual conditioning in response to a salient ethological danger signal; (4) differences in intrinsic aerobic capacity result in distinct patterns of basal gene expression in the brain as well as differences in endocrine tissue weight; and (5) circulating corticosterone levels do not differ between LCR and HCR rats under basal and unavoidable stress conditions; however, mild stress induces higher circulating corticosterone in rats with higher intrinsic aerobic capacity.

The current set of experiments suggest that HCR rats are physiologically and behaviorally 'primed' for strategies that will prepare them to respond to an acute environmental perturbation, like predation. In this way, not only do they appear more vigilant, reactive, and cautious than their LCR counterparts, their behavioral profile appears to follow a dose-response type of relationship where vigilance increases with environmental novelty, and even more so when a salient danger signal is presented. The higher levels of vigilant assessment in HCR rats are in line with a recent report showing HCR rats are more sensitive to central administration of orexin-A (Novak *et al*, 2010), considering the critical role of the orexin system in vigilance

(Chemelli *et al*, 1999). Although advantageous in some instances, these adaptations that prime HCR rats to deal with an acute stressor (ie, a cat) may negatively impact their health if stressors are chronic and unpredictable in nature, and they are unable to reduce the impact of stress via active coping (eg, exercise, escape, etc); this hypothesis has yet to be tested. In contrast to HCR, LCR rats show lower risk assessment and reactivity to acute environmental perturbations (like novelty, and cat odor) and employ a more passive coping strategy during inescapable swim stress. Although this behavioral and physiological profile would conceivably be less effective against the threat of predation, it could serve the animal better in the context of a social stress if the LCR occupies a subordinate position in the social hierarchy. Given that the LCR rats display a MetS phenotype, it is difficult to argue that they would fare 'well' in the face of chronic stress. Their disease progression, however, may be related to the intensity of the stress and their opportunity to engage in passive coping.

The behavioral responses to novelty and predator odor in HCR vs LCR rats suggest that the coping strategies differ in rats with divergent aerobic capacity depending on the anxiogenic properties or saliency of the environmental stimulus. In our first tests, we looked at behavioral responses to different paradigms that rely on environmental novelty. No differences between HCR and LCR were found in locomotor response to novelty. In addition, no statistically significant differences were found in the LDB, although a trend existed for LCR rats to have a lower latency to transition out of the light compartment ($p = 0.0532$) and for higher activity levels ($p = 0.1245$) in the light compartment compared with HCR rats.

Anecdotal differences in coping strategies were observed during handling where HCR animals exhibited much higher levels of escape behavior, struggling, and were, in general, much more aggressive and vocal. In comparison, LCR animals were very passive during handling. The behavioral patterns of HCR and LCR animals during handling are in line with reports of behavioral response to handling in animals with free access to running wheels and those housed with locked running wheels (Burghardt *et al*, 2004), respectively. Increased struggling and biting in response to handling have also been reported in wild-caught rats compared with their domesticated counterparts (Blanchard *et al*, 1986) and has been attributed to differences in defensive responding.

Differences in defensive and risk-assessment behaviors to predator odor have been reported in various rat strains (Dielenberg and McGregor, 2001), and individual differences in response to predator odor have been detected even when it was not possible to distinguish the behavior of individual rats in other paradigms like the elevated plus maze or social interaction (Hogg and File, 1994). The anecdotal observations of behavior in the HCR/LCR rats were suggestive of HCR rats displaying a more 'naturalistic' behavioral profile in response to a predator stimulus. Given the lack of differences in the locomotor response to novelty and the non-significant behavioral trends in the LDB, we decided to more specifically assess risk-assessment and defensive behaviors of LCR and HCR rats to a salient danger stimulus, cat odor. Both HCR and LCR rats exhibited higher levels of risk assessment with concurrently lower levels of

exploration while exposed to cat odor as compared with control or novel (strawberry) odors (Figure 6a and b, Table 2). Compared with LCR, HCR rats showed lower exploration in the open area during cat odor exposure, indicating that high intrinsic aerobic capacity is associated with higher levels of defensive behavior in response to a predator odor. Behavioral responses to a novel strawberry odor did not significantly differ from control odor in either HCR or LCR rats, echoing the results of the locomotor and LDB paradigms.

Interestingly, HCR rats previously exposed to cat odor showed higher levels of risk assessment and lower levels of exploration compared with HCR rats previously exposed to control odor and novel odor when returned to the context 24 h after odor exposure (Figure 6c and d, Table 2), which is indicative of contextual conditioning and has been described by a number of other groups in response to predator odors (Blanchard *et al*, 2003; Dielenberg and McGregor, 1999). This would suggest that HCR rats exposed to a predator odor made a stronger association between the predator odor and the context compared with LCR rats. The enhanced contextual conditioning of HCR rats is in line with the ability of voluntary exercise to enhance the association between salient unconditioned stimuli with environmental cues (Burghardt *et al*, 2004, 2006; Falls *et al*, 2009; Van Hooissen *et al*, 2004). This also suggests that HCR and LCR rats differ in learning. Whether differences in other forms of learning (ie, spatial, instrumental) exist among these rats will be of great interest.

Based on the associations among metabolic disorders, depression, and stress, we also examined behavioral responses of LCR and HCR rats in the FST. In this test, LCR rats exhibited higher levels of immobility compared with HCR rats and both lines of rats showed decreased immobility following treatment with desipramine. It is important to note that the difference among LCR and HCR rats existed regardless of vehicle or desipramine treatment. Further, both LCR and HCR rats showed identical patterns of immobility during the 15-min swim on day 1. Treatment with desipramine brought immobility of LCR rats to the level of HCR saline-treated rats during the 5-min swim on day 2. Both of these findings indicate that the higher levels of immobility exhibited by LCR rats on day 2 were attributable to a more passive coping strategy employed by LCRs in response to inescapable swim stress, and not to differences in the physiological capacity for physical activity. The higher levels of immobility displayed by LCRs compared with HCRs are analogous to effects reported in studies examining the effects of wheel running compared with sedentary conditions, respectively, on learned helplessness (Greenwood *et al*, 2007) and forced swimming (Bjornebekk *et al*, 2005). Given the high comorbidity among metabolic disorders (eg, cardiovascular disease, diabetes, MetS) and depression (McIntyre *et al*, 2007), these results are particularly intriguing and indicate that LCR rats may be a potential model for examining the biological linkages among these comorbid and complex diseases; however, the degree to which these findings truly model the human condition require further investigation.

The behavioral profiles of the LCR and HCR rats suggest that individual differences in inherent aerobic capacity are associated with distinct behavioral repertoires for coping

with environmental challenges. The relative success of the coping strategy is likely to depend on the type, duration, and controllability of the environmental challenge. These results are interesting when considering exercise training studies reporting that behavioral changes occur following a longer duration of exercise (in time frames similar to those required for cardiopulmonary adaptations) as opposed to the short-term physiological responses to an acute bout of exercise. This idea is supported by other studies that showed temporal effects of wheel running on behavior (Burghardt *et al*, 2004; Greenwood *et al*, 2005). Human studies also seem to support the temporally dependent response of behavior to exercise. Depressive symptomatology is reduced after a longer period of training (Dunn *et al*, 2005), and the acute effects of exercise on mood are enhanced in individuals who exercise regularly (Hoffman and Hoffman, 2008). Although there seems to be a relationship between a training response to exercise (eg, increase in aerobic capacity) with improvements in mood, it is unclear if increases in aerobic fitness are a necessity for improvements in mood (Dunn *et al*, 2001). Taken together, the behavioral profile of rats with low and high intrinsic aerobic capacity add support to the idea that affective states (ie, anxiety) may be more directly influenced by acute bouts of exercise, whereas changes in mood may require longer durations of exercise training that are associated with cardiopulmonary adaptations. Further, that HCR rats (or rats given the opportunity to exercise voluntarily) display a more 'naturalistic' phenotype compared with sedentary rats, or those with low intrinsic aerobic capacity.

As distinct coping strategies were associated with intrinsic aerobic capacity, we next investigated whether stress system function, under basal and stress conditions, associated with divergent levels of intrinsic aerobic fitness. It has long been known that circulating glucocorticoid levels are involved in the response to a variety of stressors and in regulation of energy (Tempel and Leibowitz, 1994). Further, dysregulation of the HPA stress axis is a hallmark of several forms of depression (Akil, 2005) and metabolic disorders, strengthening the bidirectional link between stress and metabolism. Circulating corticosterone levels were used to assess stress-axis function in LCR and HCR rats under several conditions. The corticosterone response following the LDB was lower in LCR rats despite similar behavioral patterns among HCR and LCR animals. To assess further the stress-axis function and capacity in these rats, we evaluated corticosterone levels at light/dark transitions and in response to inescapable cat odor. No differences were found among LCR and HCR rats for circulating corticosterone at the nadir or peak of the circadian cycle, or following unavoidable exposure to cat odor, suggesting that the response following the LDB may be mediated by higher-order limbic and stress pathways in the brain.

Differential corticosterone response to the mild stress of environmental novelty has been reported in other selectively bred rat lines that were related to distinct patterns of gene expression in the brain (Kabbaj *et al*, 2000). It is known that LCR and HCR rats have inherent differences in several neurochemical systems (Buck *et al*, 2007; Foley *et al*, 2006; Waters *et al*, 2008); however, this is the first assessment of stress-axis function in these rats. Given the differences in behavior and stress-axis activation

to environmental novelty, we sought to determine the baseline patterns of CRH, mineralocorticoid, and glucocorticoid mRNA expression in brain regions regulating stress and emotionality. In addition, we measured adrenal gland and thymus weight.

CRH is a neuropeptide-mediating HPA activation in response to stress (Bale and Vale, 2004), anxiety (Britton *et al*, 1985), and depression (Nemeroff *et al*, 1984), as well as the regulation food intake (Britton *et al*, 1982; Mastorakos and Zapanti, 2004; Richardson *et al*, 2002). CRH mRNA expression was lower in LCR compared with HCR rats, in the central nucleus of the amygdala. The comparable levels of CRH mRNA expression in the paraventricular hypothalamus among HCR and LCR rats is in line with the levels of corticosterone at the transition of light and dark. Further, the elevated expression of CRH in the CeA of HCR rats provides a potential neural basis for the elevated corticosterone response to novelty seen in the LDB, and the elevated levels of risk assessment when exposed to cat odor. Although this difference in CRH is likely not the sole cause of these behavioral differences, it may be part of the internal milieu mediating differential behavioral strategies of HCR and LCR rats in response to a threat. These findings are in line with the reports of CRH function in the amygdala, which has been implicated in behavioral response to novelty after pre-exposure to a stressor (Korte and De Boer, 2003). In this way, higher levels of CRH mRNA in HCR rats may play into their relatively higher levels of risk assessment in response to cat odor, as indicated by work in other limbic regions (Pentkowski *et al*, 2009). Amygdalar CRH has also been implicated in divergent fear-potentiated startle in selectively bred lines that do not differ in behavioral responses to environmental novelty (Yilmazer-Hanke *et al*, 2002). Further, elevated CRH in mutant mice was associated with elevated corticosterone response to stress and reduced immobility in the FST (Lu *et al*, 2008), analogous to the behavior of HCR rats in the FST and their corticosterone response to the LDB. These inherent differences in limbic and HPA axis in individuals with divergent aerobic capacity may be associated with distinct physiological resources that partially dictate the behavioral repertoire used in response to a given environmental challenge.

Basal expression of MR and GR mRNA in the hippocampus did not differ among LCR and HCR rats. Similar levels of MR mRNA in the hippocampus are not surprising as the MR regulates basal levels of glucocorticoids (Dallman *et al*, 1989; Spencer *et al*, 1998), which did not differ among HCR and LCR rats. The GR is involved in the negative-feedback regulation of the stress response (Akil, 2005), and in this case, GR mRNA did not differ among LCR and HCR rats. The similar levels of GR expression are in line with the return of circulating corticosterone to baseline levels 90 min after exposure to the LDB (Figure 3c).

Adrenal and thymus weight were lower in LCR compared with HCR rats. These results are analogous to those reported in sedentary mice (Droste *et al*, 2003) and rats (Droste *et al*, 2007) when compared with rodents engaged in wheel running, indicating that differences in autonomic output or HPA-axis function may be linked with aerobic capacity. LCR rats also had lower absolute and bodyweight-adjusted thymus weight compared with HCR rats (Figure 4b). Thymic involution is known to occur in

response to chronic stress and aging, and is believed to be related to changes in immune function that occur in response to chronic stress (Bauer *et al*, 2009). Given the difference in thymus weight, it will be intriguing to see if aerobic capacity also segregates with susceptibility to infection or longevity. The differences in adrenal and thymus weight may suggest a difference in autonomic drive between individuals with high and low aerobic capacity.

Although these studies suggest that aerobic capacity is associated with coping strategy and related neurohormonal function, an important consideration is that the treadmill screening procedure at 10 weeks of age may influence the development of these systems and behavioral strategies, as forced treadmill running to exhaustion is clearly a stressor. It could also be that these traits were inadvertently carried along during the breeding scheme. However, it is known that individual rats strains show a wide range of intrinsic aerobic capacity (Barbato *et al*, 1998) and that some of these same in-bred rat strains (eg, Copenhagen vs Fischer) show inherent differences in behavior (Wilhelm and Mitchell, 2009). Further, human studies have supported a role for changes in aerobic capacity via exercise with improvements in mood and affect (Dunn *et al*, 2005; Herring *et al*, 2010; Hoffman and Hoffman, 2008). With these differences in mind, it would seem that aerobic capacity is a strong predictor of behavioral and neurohormonal responses to environmental challenges. Nonetheless, future experiments are planned to address this issue by comparing animals screened for treadmill running behavior at 10 weeks of age with those who are not screened on the treadmill.

Taken together, these experiments show that rats born with low intrinsic aerobic capacity, and consequently low fitness, show decreased risk assessment in response to salient danger signals and passive coping in a test of antidepressant efficacy. In contrast, rats with high intrinsic aerobic capacity and fitness are more vigilant and cautious in response to environmental novelty and salient danger signals and show a more active coping strategy in a test of antidepressant efficacy. Coupled with their distinct metabolic and neuroendocrine profiles, the contrasting behavioral phenotypes of the LCR and HCR rats provide a unique model system for studying the relationship between metabolism and coping strategies that buffer against stress.

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DISCLOSURE

The authors declare no conflicts of interest.

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