

Voluntary Exercise Impacts on the Rat Hypothalamic-Pituitary-Adrenocortical Axis Mainly at the Adrenal Level

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Key Words

Voluntary exercise · HPA axis · Hippocampus ·
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Abstract

Introduction: Evidence is accumulating that the regular performance of exercise is beneficial for stress coping. However, the hypothalamic-pituitary-adrenocortical (HPA) axis of voluntarily exercising rats has never been comprehensively investigated. **Methods:** Therefore, male Sprague-Dawley rats were given access to a running wheel in their home cage for 4 weeks in which they ran 4–7 km per night. **Results:** After 4 weeks, the exercising animals showed significantly less body weight gain, less abdominal fat tissue, decreased thymus weight, and increased adrenal weight (relative to body weight). Furthermore, tyrosine hydroxylase (TH) mRNA levels were selectively increased in the right adrenal medulla indicating an increase in sympathoadrenomedullary capacity in exercising rats. No changes were observed in paraventricular corticotropin-releasing hormone (CRH), arginine-vasopressin (AVP) and oxytocin mRNA levels. Mineralocorticoid receptor (MR) mRNA levels in hippocampus and glucocorticoid receptor (GR) mRNA levels in frontal cortex, parvocellular paraventricular nucleus and anterior pituitary were unchanged, whereas GR mRNA levels were increased in distinct hippocampal cell layers. Early morning baseline levels of plasma ACTH and corticosterone were similar in

both groups. Interestingly, the response to different stressful stimuli (e.g. forced swimming, novelty) revealed that the exercising rats showed stressor-specific changes in HPA hormone responses. Forced swimming evoked a markedly enhanced response in corticosterone levels in the exercising rats. In contrast, if rats were exposed to a novel environment, exercising rats showed a much lower response in corticosterone than the control animals. However, the response in ACTH to either stressor was comparable between groups. Thus, in exercising rats physically demanding stressors evoke enhanced glucocorticoid responses whereas mild psychologically stressful stimuli such as novelty result in an attenuated glucocorticoid response. Interestingly, this attenuated hormone response corresponded with the observation that the exercising rats showed less anxious behaviour in the novelty situation. **Conclusions:** The differential responses in plasma corticosterone levels to different types of stress in the face of comparable responses in ACTH levels underscore the existence of critical regulatory control mechanisms at the level of the adrenal gland. We have hypothesized that changes in the sympathoadrenomedullary input may play an important role in these distinct glucocorticoid responses to stress. Our previous studies have shown similar changes in voluntarily exercising mice. Therefore, we conclude that the effects of exercise on the organism are not species-specific. Thus, our observations may have translational implications for the human situation. Copyright © 2007 S. Karger AG, Basel

Introduction

The regular performance of exercise produces profoundly beneficial effects in terms of physical and mental health and fitness. Exercise has been shown to improve autonomic control, metabolism and immune function [1–6]. Moreover, evidence is accumulating favouring the application of exercise as an effective co-treatment for anxious and depressed patients, in addition to drug and behavioural therapy [7–10]. Physiological hallmarks of these mood disorders include impaired control of the HPA axis and sympathetic nervous system culminating in hypersecretion of glucocorticoid hormones and adrenaline/noradrenaline at baseline [11–14]. Such out-of-context hypersecretion of these hormones is potentially harmful to the organism contributing largely to the observed psychiatric and psychosomatic symptoms and comorbidities (e.g. cardiovascular disease, metabolic syndrome, obesity) seen in these patients [12, 15, 16]. The reason for the disturbed hormonal homeostasis is currently unknown. The hypersecretion of glucocorticoid hormones could at least in part be a consequence of enhanced baseline adrenomedullary adrenaline secretion, but also this is unknown.

Our recent published work on long-term voluntarily exercising mice shows that exercise benefits appropriate glucocorticoid responses to stressful events [17, 18], decreases anxiety and impulsiveness [19], and improves sleep quality [20]. Based on these data, we have hypothesised that the exercise-evoked changes in glucocorticoid responses to stress are the result of alterations in sympathoadrenomedullary control of glucocorticoid secretion, at least in the mouse. Insight into these mechanisms is not only important for our general understanding of the neuroendocrine and stress physiological implications of exercise but also to gain insight into how the sympathoadrenomedullary system contributes to shaping the adrenocortical glucocorticoid response to different types of stress. These studies contribute to resolving the sympatho-glucocorticoid disturbances seen in stress-related psychiatric and psychosomatic disorders.

The present experiments aimed to determine the effects of long-term voluntary exercise on the rat HPA axis activity under baseline conditions and in response to stressors with different physical impact. The obtained data would help to clarify whether our previously collected exercise data in mice were specific for this species or whether they are warranted across species. This is important for translation of data to the human situation be it in health or disease. Furthermore, such data would

open avenues of research which are currently not possible in mice, such as for instance experimental approaches involving repeated blood sampling via jugular vein cannulas over extended periods of time.

Here, we show that in the HPA axis of rats long-term voluntary exercise mainly exerts effects at the adrenal gland level possibly involving changes in sympathoadrenomedullary input. These effects were similar to those found in mice. Furthermore, we show that in exercised rats the glucocorticoid response to novel cage exposure is attenuated presumably because this event may be less fearful for the exercised animals.

Materials and Methods

Animals

Male Sprague-Dawley (SD) rats (140–160 g) were singly housed under standard lighting (14:10-hour light/dark cycle), humidity (50–60%) and temperature (22–23°C) conditions. Food and water were available *ad libitum*.

Voluntary Exercise Paradigm

After habituation to the housing conditions for 5 days, the experimental group was allowed free access to a running wheel (diameter 34 cm) in their home cages for a period of four weeks. Using an infrared video camera and a wheel-turning counting system, it was observed that the rats were mainly running in the wheel during the dark phase of the diurnal cycle. The rats ran approximately 4–7 km per night which is in agreement with other reports [21, 22]. The housing of the sedentary (i.e. control) animals remained unchanged. All animal experiments were approved by the UK Home Office. Voluntary wheel running is not regarded as a form of stereotypic behaviour [23] because it is not expressed at the cost of resting behaviour, i.e. sleep [20], as is the case with other reported locomotor stereotypies [24, 25].

Physical Parameters

The animals were weighed weekly, and also food intake was determined weekly. Water intake was determined once a week over a 24-hour period. After the experimental (exercise) period, the animals were killed, and the abdominal (i.e. peritoneal + perirenal) adipose tissue, thymus, pituitary gland and adrenal glands were dissected and weighed.

In a separate experiment, the adrenal glands were quickly frozen on dry ice, cut into 12- μ m cross sections in a cryostat, mounted on slides (Superfrost, Fisher Scientific, Loughborough, UK) previously coated with poly-L-lysine, fixed in a modified Carnoy's solution (60% ethanol/40% glacial acetic acid) and stained with Mayer's hemalum solution and counterstained with 0.1% eosin Y (Sigma). After dyeing, the sections were dehydrated and cleared in ethanol, mounted in Histomount (National Diagnostics, Fisher), and coverslipped. The quantitative analysis of area measures was performed as described previously [17] by using a video image analysis system (Scion image, NIH). The area of the complete adrenal cross section as well as that of the medullar part were determined by direct measurement, whereas the area of the cortical

part was estimated by subtraction of the medullar area from the total adrenal area. As before [17, 18], only sections were analyzed from the mid part of the adrenal gland. Areas are expressed as the number of square pixels. Areas determined for the two adrenals of an animal are presented separately for the left and the right adrenal as well as added to obtain one value for the two adrenals together for the total, cortical and medullar area per animal's adrenal gland. Subsequently, mean values \pm SEM were calculated ($n = 5-6$ per experimental group) and corrected for bodyweights.

Neuroendocrine Experiments

The neuroendocrine experiments were conducted after 4 weeks of voluntary exercise or non-exercise, i.e. the sedentary control condition. All experiments were performed between 07.00 and 11.00 h a.m. For assessment of baseline plasma hormone levels, individual rats were quickly anaesthetised (<15 s) in a glass jar containing saturated isoflurane (Merial Animal Health Ltd., UK) vapour, after which the animals were decapitated immediately. Trunk blood was collected in ice-chilled EDTA-coated tubes (15 ml) containing 50 μ g aprotinin (Trasyolol, Bayer). As shown previously, this procedure yields baseline plasma ACTH and corticosterone data [26-28].

For determination of stress-induced plasma ACTH and corticosterone concentrations, the rats were either exposed to a novel environment or submitted to a forced swimming procedure. For the novel environment exposure, control or exercised rats were placed singly in new cages containing clean sawdust for 30 min in an experimental room illuminated at 400 lx (holding room light: 80-100 lx). Except for the light condition, the experimental room had the same environmental (i.e. humidity) conditions as the holding room. Next, the rats were quickly anaesthetised, decapitated, and trunk blood was collected as described above. Furthermore, the behaviour during the time in the novel cage was recorded for behavioural analysis using a CCTV digital camera system and hard disk recorder. For forced swimming, the rats were placed singly for 15 min in a glass beaker containing water at 25°C. The water depth was 20 cm, allowing the rat to touch the bottom of the glass with the tip of its tail. After completion of the swimming procedure, the animals were rapidly dried with a towel, quickly anaesthetised, decapitated, and trunk blood was collected as described above.

Plasma samples for ACTH and corticosterone measurement by RIA (ICN Biomedicals, Costa Mesa, Calif., USA) were stored at -80 and -20°C, respectively. The inter- and intra-assay coefficients of variance for ACTH were 7 and 5%, respectively, with a detection limit of 2 pg/ml. For corticosterone, the inter- and intra-assay coefficients of variance were 7 and 4%, respectively, with a detection limit of 0.4 ng/ml.

Behavioural Analysis

The different behaviours of the experimental animals during the novel environment exposure (i.e. stationary (not moving in a standing or sitting position), lying/sleeping, walking, rearing, grooming, scratching and burrowing) were scored after each 10 s over the whole 30-min experimental period and the mean score \pm SEM for the whole time period was calculated.

In situ Hybridization Histochemistry

Under early morning resting conditions, rats were killed as described above. The whole brains were quickly removed, snap-

frozen in isopentane at -40°C and deep-frozen in dry ice. Twelve-micron-thick coronal brain sections at the level of the hypothalamic paraventricular nucleus (PVN; from bregma level -0.70 to -0.22 mm), the dorsal (from bregma level -2.92 to -3.96 mm) and ventral hippocampus (from bregma -4.80 to -5.30 mm), the medial prefrontal cortex mPFC region including the infralimbic cortex (IL; from bregma level 3.20 to 2.20 mm), the granular insular cortex (GI; from bregma 1.20 to 0.20 mm), and the pituitary were cut in a cryostat and mounted on glass slides (Super frost plus, Fisher). Also the adrenal glands were collected, frozen on dry ice, cut into 12- μ m-thick sections in a cryostat, and mounted on Super frost glass slides.

The in situ hybridization assays for CRH, AVP and oxytocin mRNA in the PVN, tyrosine hydroxylase (TH) mRNA in the adrenal medulla, and proopiomelanocortin (POMC) mRNA in the anterior pituitary were carried out using oligodeoxynucleotide probes, as reported previously [17, 18]. For each oligodeoxynucleotide probe sections of all experimental groups were run in the same assay under identical conditions. Briefly, the oligodeoxynucleotides were labelled at the 3'-end with α [³⁵S]-dATP (NEN, Boston, Mass., USA) using terminal deoxynucleotidyl transferase (Roche Diagnostics). Radiolabeled probe (10⁶ cpm/200 μ l/slide) was diluted into hybridization buffer (consisting of 1 \times Denhardt's solution, 0.25 mg/ml yeast transfer RNA, 0.5 mg/ml salmon sperm DNA, 10% dextran sulfate, 10 mM dithiothreitol (DTT) and 50% formamide (all from Sigma), applied to the slides and incubated for 20 h at 45°C. After hybridization, the slides were washed four times in 1 \times SSC at 45°C for 15 min, dehydrated in ethanol, air-dried and exposed to autoradiography film (Kodak Biomax MR-1, Kodak, N.Y., USA) for 3-5 days for TH mRNA and POMC mRNA and 7-10 days for CRF mRNA, oxytocin mRNA and AVP mRNA.

The in situ hybridization histochemistry for the detection of MR and GR mRNA was performed using ³⁵S-labeled antisense cRNA probes. Techniques for probe synthesis, hybridization, and autoradiographic localization of mRNA signal were adapted from Simmons et al. [29]. Briefly, the MR and GR probes (a gift from Prof. Jonathan R. Seckl, University of Edinburgh) were linearized with *Hind*III or *Ava*I, respectively (for generation of the antisense probe; Roche Diagnostics), or *Eco*RI (sense probes; Roche Diagnostics). The MR and GR cRNA probes were generated using the SP6/T7 polymerase Hybridisation Kit (Roche Diagnostics). The used radiolabel was α [³⁵S]-UTP (NEN). Shortly before hybridization, the sections were air-dried at room temperature (RT), fixed in 4% formaldehyde for 10 min at RT, washed three times in PBS, and then acetylated in 0.25% acetic anhydride in 0.1 M triethanolamine for 10 min at RT. Sections were dehydrated in serial ethanol solutions, defatted in chloroform and allowed to dry. Subsequently, the sections were hybridized overnight at 58°C in a solution containing 50% formamide, 20 mM Tris-HCl pH 8.0, 300 mM NaCl, 5 mM EDTA, 10% dextran sulphate, 0.02% Ficoll 400, 0.02% polyvinylpyrrolidone, 0.02% BSA (all from Sigma), 0.5 mg/ml tRNA (Roche Diagnostics), 200 mM DTT (Sigma) and the ³⁵S-labeled MR or GR cRNA probe (5 \times 10⁶ cpm/ μ l), after which they were treated with 20 μ g/ml RNase A for 20 min at 37°C. Next, the sections were washed in 2 \times SSC (15 mM NaCl/1.5 mM sodium citrate pH 8.0)/1 mM DTT for 10 min at RT, 1 \times SSC/1 mM DTT for 10 min, 0.5 \times SSC/1 mM DTT for 10 min, twice in 0.1 \times SSC/1 mM DTT at 64°C for 30 min, and, finally for 10 min in 0.1 \times SSC. Sections were then dehydrated in serial ethanol/ammonium acetate solutions, air-dried and exposed to autoradiography film

(Kodak Biomax MR-1, Kodak, N.Y., USA) for 2–4 weeks depending on probe and region.

Specificity of hybridization/probes was ensured by preparing ³⁵S-labelled sense RNA probes of similar specific activity and determining hybridization under identical conditions. No specific cellular hybridization signal was seen using any of the sense probes (data not shown).

Optical Densitometry

Representative autoradiographic images of the region of interest were digitally recorded using a CCD video camera (XC-77CE, Sony). Semi-quantitative analyses of mRNA levels were performed blind using a densitometric video image analysis system (Scion Image, NIH). The optical density (grey values, expressed as arbitrary units; resolution: 256 levels) of an area encompassing the region of interest was determined and the background (measured just outside the area of interest in an area containing no apparent hybridization signal) was subtracted. MR and GR mRNA levels were measured in the various subfields of the dorsal and ventral hippocampus, GR mRNA levels in the IL and GI, AVP and OXT mRNA levels in the magnocellular part of the PVN, AVP, OXT, CRH and GR mRNA levels in the parvocellular part of the PVN, and POMC and GR mRNA levels were measured in the anterior lobe of the pituitary; the intermediate (and posterior) lobe were excluded from analysis. From each animal, at least 3 sections of a representative assay were analyzed. The optical density data are presented as net grey values (mean ± SEM of 4–8 rats/group).

Regarding the determination of TH mRNA expression in the adrenal medulla, only sections stemming from the middle part of the adrenal gland were used for the *in situ* hybridization assays. Three sections per adrenal gland were used. Autoradiograms of all sections were densitometrically analyzed for TH mRNA levels providing a group means ± SEM values for the left and right adrenal medullas separately. As conducted previously [17, 18], we determined an integrated optical density of TH mRNA expression in the left and right adrenal medulla by multiplying the optical density value with the respective measure of the medullar area (area expressed as square pixels/1,000). Thus, the unit of integrated optical density is: net grey value × square pixels/1,000.

Statistical Analysis

The data on body weight and food and water intake were tested for statistically significant differences using two-way analysis of variance (ANOVA) with repeated measures followed in appropriate cases by post-hoc tests with contrasts. The experimental data on the adrenal, pituitary and thymus weights, adrenal size, hormone levels, behaviour and mRNA levels were tested with Student's *t* test or with two-way ANOVA followed in appropriate cases by post-hoc tests with contrasts. As level of signifi-

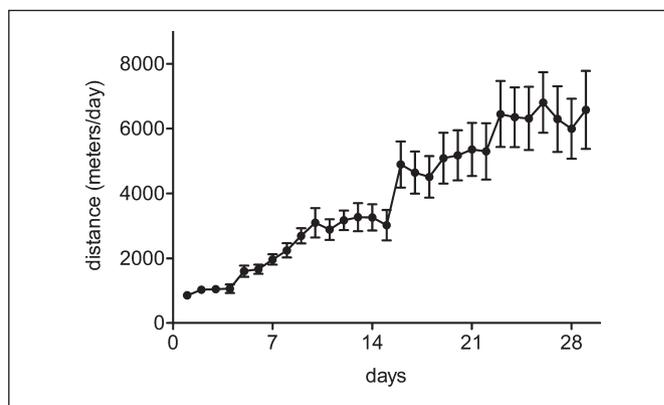


Fig. 1. Time course of wheel running activity of male Sprague-Dawley rats over 4 weeks (n = 12, mean values ± SEM).

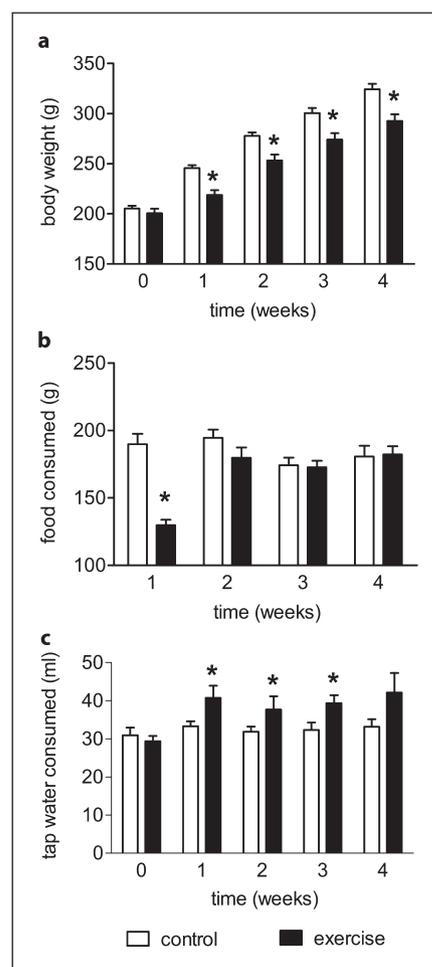


Fig. 2. Changes in (a) body weight (time course; control, n = 12; voluntary exercise, n = 12) (b) food intake and (c) water intake in exercised and control rats over a 4-week time period. Data are expressed as means ± SEM. The exercising rats had significantly less body weight gain than the control animals and there was a

significantly lower food intake in the exercising animals but only in the first week (* *p* < 0.05). The water intake was significantly higher in the exercising rats from the first week onward. For ANOVA analysis, see text. * Significantly different from control rats at the respective time point (post-hoc tests with contrasts).

Table 1. Changes in physical parameters after long-term voluntary exercise in rats

| | Body weight | Abdominal fat | Thymus | Adrenals | Pituitary |
|----------------------------|-------------|---------------|---------------|-----------------|-----------------|
| Absolute weight | | | | | |
| Control | 318 ± 6 | 274 ± 1 | 482 ± 18 | 49 ± 1 | 6.5 ± 0.2 |
| Exercise | 295 ± 5* | 195 ± 1* | 364 ± 16* | 50 ± 1 | 5.1 ± 0.4* |
| Relative to body weight, % | | | | | |
| Control | | 0.86 ± 0.03 | 0.15 ± 0.005 | 0.015 ± 0.0004 | 0.0020 ± 0.0001 |
| Exercise | | 0.66 ± 0.02* | 0.12 ± 0.004* | 0.017 ± 0.0004* | 0.0017 ± 0.0001 |

Body and organ weights expressed as absolute (body weights in grams, other in milligrams) and/or relative to body weight (% of body weight) values. Tissues were collected from 4-weeks voluntarily exercised rats and control sedentary rats. Data are expressed as (mean ± SEM, n = 12; pituitary data: n = 6). * p < 0.05, Student's t test.

cance, p < 0.05 was accepted. For all post-hoc tests with contrasts, the level of significance was reduced according to the Bonferroni procedure to keep the probability of a type 1 error less than 5%.

Results

Wheel Running Performance

Figure 1 shows the daily running distances of male Sprague-Dawley rats over a period of 4 weeks. The rats showed an increasing running performance which appeared to stabilize after approximately 16 days and stayed at this level (i.e. 5.7 ± 0.2 km/day; mean ± SEM; n = 12) for the remainder of the time.

Body Weight and Food and Water Intake

Figure 2 shows a time course of the body weight gain (fig. 2a) and food (fig. 2b) and water intake (fig. 2c) of control and exercising rats over the 4-weeks period. Both groups of rats gained weight over the 4-week time period, but this was significantly less in the exercising animals (fig. 2a; effect of exercise: F(1,22) = 12.5, p < 0.05; effect of time: F(4,88) = 675, p < 0.0001; interaction time × exercise: F(4,88) = 10.4, p < 0.0001). As shown in figure 2b, exercising animals consumed less food selectively in the first week (effect of exercise: F(1,22) = 5.75, p < 0.05); interaction of time × exercise: F(3,66) = 27.9, p < 0.0001, a phenomenon which has been reported before [21, 22]. From the second week onward there were no differences in food intake observed anymore. The exercising rats showed an overall increased water intake which changed over time (fig. 2c; effect of exercise: F(1,18) = 4.62, p < 0.05; effect of time: F(4,72) = 9.7, p < 0.0001; interaction time × exercise: F(4,72) = 6.3, p < 0.0001).

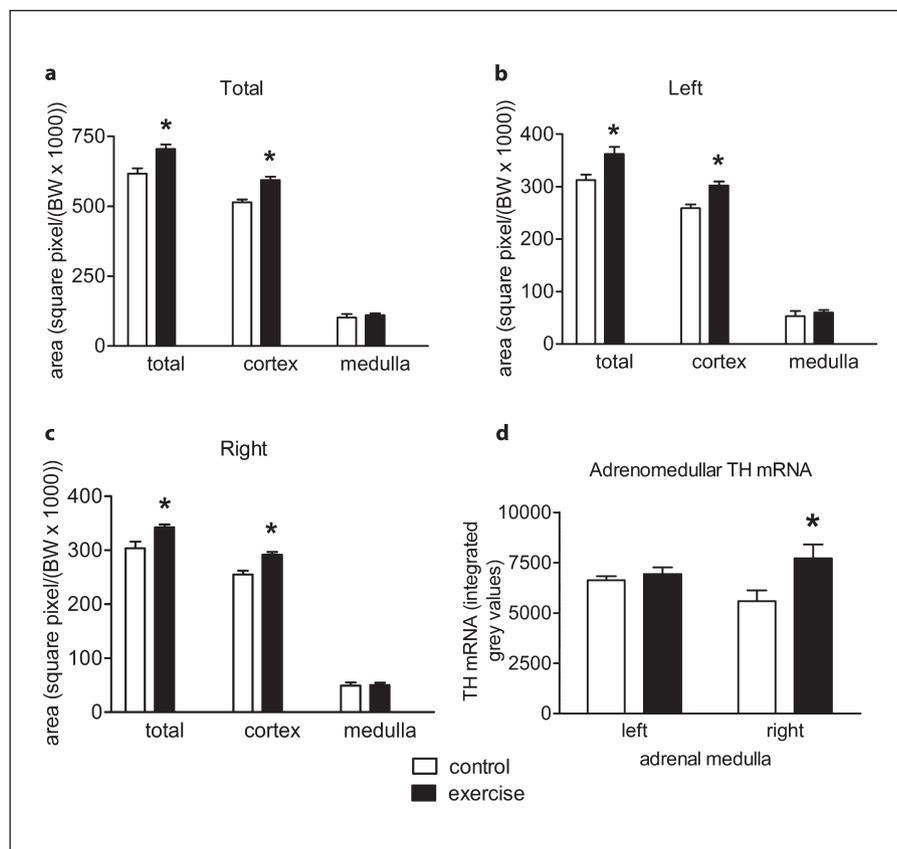
Physical Parameters: Peritoneal Fat, Thymus and Adrenal Weight

By the end of the 4-week experimental period, the body composition was different between the groups. Table 1 shows the measurements of the peritoneal fat tissue, the thymus, the pituitary and the adrenal glands expressed as absolute weights and calculated as percent of body weight. This calculation was conducted because there were significant differences in body weights between the experimental groups. The exercised rats showed substantially less abdominal fat tissue than the control rats in both absolute and relative-to-body-weight values (table 1). The thymus weight was reduced as well in the exercised rats (both absolute and relative weights) whereas the adrenal weights, if calculated as percentage of body weight, were increased after exercise. Together the thymus and adrenal weight data indicate that the activity of the HPA axis was, at least transiently, elevated during the experimental period [30], presumably during running [17]. Exercised rats had smaller pituitaries than the controls, a difference which was lost when relating the pituitary weights to the respective body weights (table 1).

Physical Parameters: Changes in the Adrenal Gland

To assess whether 4 weeks of voluntary exercise evokes changes in the composition of the adrenal glands (i.e. cortex vs. medulla), area measures of the cortical and medullar part in adrenal cross sections were measured (fig. 3). Initially we determined the entire area of cortical and medullar cross sections of the two adrenals together for each rat (fig. 3a: 'entire: left + right') for sake of presenting an 'entire' cortical and medullary capacity. This analysis showed that the entire area of the adrenal cortex is significantly increased in exercising rats (Student's t test, p < 0.05), which also resulted in an increased total (i.e.

Fig. 3. Changes in the size and medullary TH mRNA expression of the left and right adrenal gland in exercised (4 weeks) and control rats. Quantitative measurements of areas by computerized image analysis of either the entire (i.e. left + right) adrenal surface (a) or the left (b) and the right (c) adrenal gland separately. The total surface area and the medullary area were measured and, by subtraction, the cortical area was determined (a–c). d TH mRNA was detected by in situ hybridization histochemistry and autoradiograms were analyzed by computerized image analysis. TH mRNA levels are expressed as integrated optical density (i.e. net grey values \times square pixels/1,000). For more details, see ‘Materials and Methods’ and ‘Results’. Data are presented as means \pm SEM (n = 5–6). * Significant difference between exercise and control (post-hoc tests with contrasts).



cortex + medulla) adrenal area (Student's t test, $p < 0.05$; fig. 3a, 'total'), whereas the adrenomedullary area was not significantly changed. Next, we analyzed the two adrenals separately (see fig. 3b 'left' and fig. 3c 'right'). For the total area, overall the area of the left adrenal was significantly larger than that of the right one (effect of left vs. right: $F(1,54) = 4.17$, $p < 0.05$; fig. 3b, c). Four weeks of voluntary exercise resulted in a selective increase in the size of both left and right adrenal cortices whereas no changes were observed in the adrenal medullas (effect of exercise: $F(1,54) = 37.95$, $p < 0.05$; asterisks: $p < 0.05$, post-hoc tests with contrasts; fig. 3b, c).

TH mRNA Expression in the Adrenal Medulla

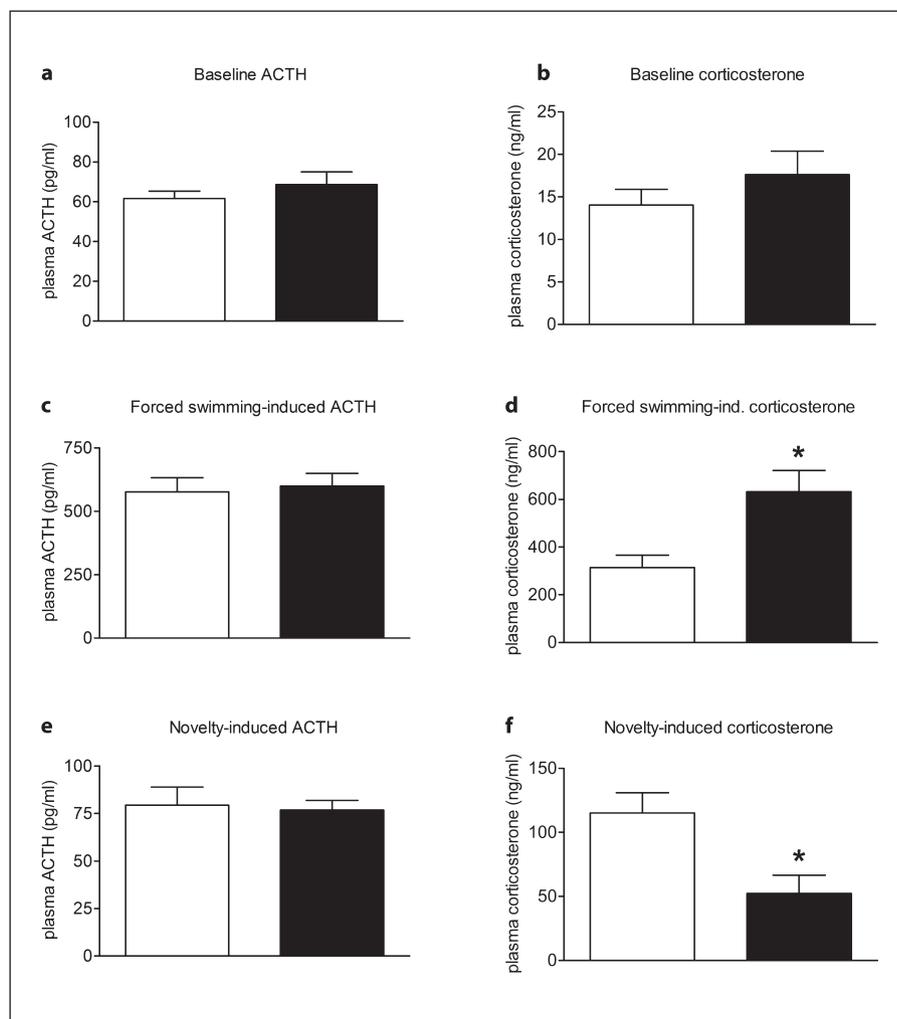
TH mRNA expression in the adrenal medulla was measured as an index for sympatho-adrenomedullary capacity [31]. The sympathoadrenomedullary input is a well-known modulator of the sensitivity of the adrenal cortex for ACTH [32]. Exercise evoked an overall increase in TH mRNA expression in the adrenal medulla (fig. 3d; effect of exercise: $F(1,18) = 11.11$, $p < 0.05$; interaction of exercise \times left/right: $F(1,18) = 2.2$, NS), but a significant

increase in TH mRNA levels was only observed in the right medulla after exercise ($p < 0.05$, post-hoc tests with contrasts).

Plasma ACTH and Corticosterone Measurements

Figure 4 shows the plasma levels of ACTH and corticosterone under early morning baseline conditions (fig. 4a, b) and in response to two different types of stress (fig. 4c–f). The early morning baseline levels of ACTH and corticosterone were similar between the two groups. However, there were distinct responses to the different types of stress. In response to forced swimming, the exercised rats showed an enhanced plasma corticosterone response as compared to that in the sedentary control animals (fig. 4d). In contrast, exposure to a novel environment resulted in corticosterone levels in the exercised rats which were more than 50% lower than those in the control animals (fig. 4f). Remarkably, the ACTH levels in response to the different stressors were comparable between groups (fig. 4c, e).

Fig. 4. Early morning baseline plasma ACTH (**a**) and corticosterone (**b**) levels (presented as means \pm SEM) in control rats and voluntarily exercised rats after a 4-week time period (n = 11–12). The plasma ACTH and corticosterone levels were similar in both groups. **c, d** ACTH and corticosterone levels of rats killed immediately after 15 min of forced swimming. Exercised rats showed similar rises in plasma ACTH levels as the control animals, but the rises in plasma corticosterone levels were significantly higher than those in the controls (n = 5–6). **e, f** Plasma ACTH and corticosterone levels in control and exercised rats killed after 30-min novel environment stress (i.e. new cage). Exercised rats showed similar rises in plasma ACTH levels as the control animals (**e**), whereas plasma corticosterone levels were significantly lower than those in the controls (**f**). * $p < 0.05$, Student's t test, means \pm SEM, n = 5–6 for both groups.



Corticosteroid Receptor mRNA Expression

To explore whether changes in corticosteroid receptor expression may be involved in the altered HPA responses observed in the exercised rats, we conducted in situ hybridization histochemistry regarding MR and GR mRNA for various brain regions and the anterior pituitary of control and exercised rats (table 2). We observed no significant differences between control and exercised rats for either MR or GR mRNA in any of the measured regions except for GR mRNA levels in the hippocampus. Hippocampal GR mRNA levels were significantly increased in the dorsal CA1, CA2 and CA3 subregions, and in the ventral CA3 subregion, whereas in the dorsal dentate gyrus and ventral CA1 a trend towards an increase in GR mRNA levels was found (table 3).

Neuropeptide mRNA Expression in the Hypothalamic Paraventricular Nucleus

To determine whether changes in neuropeptide expression in the hypothalamic may be involved in the altered HPA axis responses observed in the exercised rats, we conducted in situ hybridization histochemistry for CRH, AVP and OXT mRNA levels in the PVN of control and exercised rats. We observed no significant differences in CRH, AVP or OXT mRNA expression levels between groups in neither the magno- nor the parvocellular part of the PVN (table 3).

POMC mRNA Expression in the Anterior Pituitary

To further elucidate changes at the level of the pituitary, changes in POMC mRNA levels were examined in the anterior pituitary lobe of exercised and control rats. Surprisingly, POMC mRNA levels in the anterior pitu-

Table 2. Glucocorticoid (GR) and mineralocorticoid (MR) receptor mRNA expression in brain and pituitary

| Region | GR | | MR | |
|-----------------------------|---------|--------------------------|---------|----------|
| | control | exercise | control | exercise |
| <i>Hippocampus</i> | | | | |
| Dorsal part | | | | |
| Subiculum | 63 ± 5 | 76 ± 6 | 71 ± 14 | 64 ± 11 |
| CA1 | 114 ± 6 | 130 ± 2* | 117 ± 7 | 124 ± 4 |
| CA2 | 127 ± 4 | 142 ± 2* | 120 ± 8 | 122 ± 6 |
| CA3 | 64 ± 3 | 80 ± 3* | 112 ± 6 | 111 ± 3 |
| Dentate gyrus | 118 ± 4 | 128 ± 3 ^{0.069} | 128 ± 7 | 138 ± 5 |
| Ventral part | | | | |
| Subiculum | 51 ± 7 | 57 ± 4 | 76 ± 4 | 72 ± 3 |
| CA1 | 82 ± 9 | 104 ± 6 ^{0.071} | 98 ± 8 | 104 ± 5 |
| CA3 | 63 ± 4 | 78 ± 4* | 107 ± 7 | 117 ± 5 |
| Dentate gyrus | 94 ± 6 | 86 ± 7 | 106 ± 5 | 114 ± 6 |
| <i>Frontal cortex</i> | | | | |
| Infralimbic cortex, IL | 41 ± 6 | 47 ± 3 | | |
| Granular insular cortex, GI | | | | |
| | 61 ± 4 | 70 ± 4 | | |
| <i>PVN</i> | | | | |
| Parvocellular part | | | | |
| | 24 ± 1 | 24 ± 2 | | |
| <i>Pituitary</i> | | | | |
| Anterior part | | | | |
| | 33 ± 3 | 29 ± 1 | | |

MR and GR mRNA levels in various brain regions and in the pituitary of 4-weeks exercised and control rats. The mRNA levels were determined by in situ hybridization histochemistry and semi-quantitative computerized image analysis of the autoradiographic films (for details, see 'Materials and Methods'). Data are expressed as net grey values (means ± SEM, n = 5–6). * p < 0.05, Student's t test; in case of a trend, the p values are given in superscript.

Table 3. Corticotrophin-releasing hormone (CRH), vasopressin (AVP) and oxytocin (OXT) mRNA expression in the hypothalamic paraventricular nucleus (PVN)

| | Parvocellular part | | Magnocellular part | |
|-----|--------------------|----------|--------------------|----------|
| | control | exercise | control | exercise |
| CRH | 149 ± 3 | 152 ± 1 | | |
| AVP | 147 ± 3 | 147 ± 2 | 165 ± 1 | 163 ± 1 |
| OXT | 147 ± 4 | 138 ± 3 | 145 ± 4 | 145 ± 4 |

CRH, AVP and oxytocin mRNA levels in the parvocellular and the magnocellular part of the PVN in 4-weeks exercised and control rats. The mRNA levels were determined by in situ hybridization histochemistry and semi-quantitative computerized image analysis of the autoradiographic films (for details, see 'Materials and Methods'). Data are expressed as net grey values (means ± SEM, n = 5–7). No significant differences were observed.

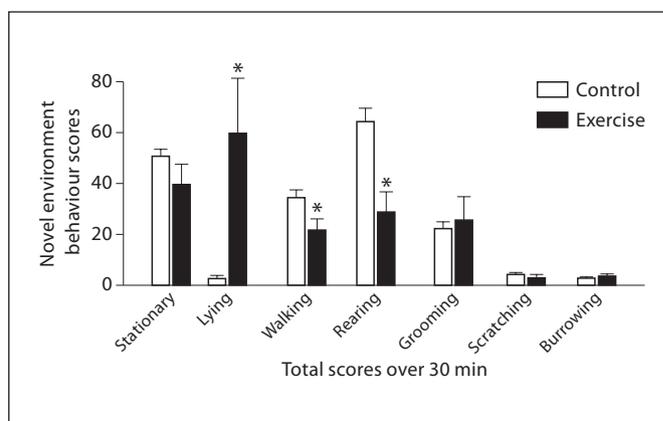


Fig. 5. Behavioural scoring of control and exercised rats (means ± SEM, n = 6 for both groups) during the 30-min novel environment exposure (i.e. new cage). The exercised animals displayed significantly more resting behaviour, whereas the control rats displayed more active exploratory (rearing and walking) behaviour. * p < 0.05, Student's t test.

ity were significantly elevated in the exercised rats (28 ± 2.7 vs. 37 ± 1.5; p < 0.05, Student's t test). However, given that the exercised rats had smaller pituitaries, presently it cannot be excluded that the increase in POMC gene expression is due to a higher density of POMC mRNA-expressing corticotrophic cells in these pituitaries rather than an increased expression of POMC per cell.

Behaviour of Exercised and Control Rats during Novel Cage Exposure

Figure 5 shows the behaviour of the exercised and control rats displayed during their 30-min stay in a novel cage. These are data of the same animals whose plasma ACTH and corticosterone levels were shown previously in figure 4e and f, respectively, i.e. in blood collected at the end of the 30-min exposure time. The exercised rats showed less active exploratory behaviours (walking and rearing) than the sedentary controls but, in contrast, lied much more down to rest or sleep. This enhanced resting/sleeping behaviour was primarily displayed during the second half of the 30-min novel cage exposure (data not shown). The parameters stationary, grooming, scratching and burrowing were not significantly different between the two groups (fig. 5).

Discussion

In the present study, we show that 4 weeks of voluntary exercise results in marked physical and physiological changes in rats. Exercised rats showed lower body weights and less abdominal fat tissue than the sedentary controls. Regarding changes in the HPA axis, both macroscopic and functional changes point to the adrenal gland as the part of the HPA axis where effects of exercise appear to converge.

The Sprague-Dawley rats ran approximately 6 km per day which they performed almost completely during the nighttime. The exercising rats (in contrast to exercising mice [17, 18] gained significantly less weight than the sedentary controls which may be due to the decreased food intake in the initial running phase. These observations are in agreement with other reports [21, 22, 33]. After normalization of food intake, the body weight curves of the sedentary and exercising rats developed in parallel. Furthermore, the changes in body composition of the exercising animals may be attributable to substantial loss of abdominal adipose tissue [17, 18, 34, 35, present study], skeletal muscle enlargements [36] and increases in heart weight [35, 36]. The reduction in adipose tissue is a result of the enhanced lipolysis in these animals [34]. The increased water consumption in the exercising rats may be due to enhanced evaporation because of the, to be expected, increases in body temperature and breathing frequency during exercise. Previously, this phenomenon has already been observed in exercising mice [17, 18].

The exercising rats presented increased adrenal weights and decreased thymus weights, which is consistent with episodically elevated glucocorticoid levels, i.e. during the first half of the dark phase, the time when the animals are running [17]. Increased adrenal weights after exercise is a long-known finding [37–39]. Furthermore, the increase in adrenal weight corresponded with the observed enlargement of the adrenal cortex in the exercised rats. In contrast to our earlier observations in exercised mice [17, 18], exercised rats showed enlargement of both the left and right adrenal cortices. The exercised mice showed only an increase in the right adrenal cortex; the left one remained unchanged [17, 18]. We hypothesized at that time that the selective increase of the right adrenal cortex may be due to the risen sympathetic capacity in the medulla of specifically this adrenal gland [17, 18, 40]. However, in exercised rats both adrenal cortices were enlarged despite an increase in sympathetic capacity only in the right adrenal medulla. Thus, it appears that mechanisms other than or in addition to the sympathoadreno-

medullary input are governing adrenal cortex size in exercising animals. Presently, species-specific differences between mice and rats in adrenocortical control cannot be excluded as well.

In contrast to our observations, other researchers observed substantially enlarged adrenal medullas in animals and man after exercise training [41–43]. However, in those cases subjects were subjected to either high-intensity exhaustive exercise (cf. man [41]) or forced to run in a treadmill usually on a cumulative performance schedule [42–44]. These observations further underscore that the voluntary exercise paradigm is not comparable with those models and that exhaustive exercise induces changes which go beyond the ones seen in either mice [17, 18] or rats [present study] after voluntary exercise.

The exercised and sedentary control rats showed markedly different behaviour in the 30-min novel cage exposure. The control rats explored the cage virtually continuously and hardly lied down to rest. Overall, the exercised rats showed less exploratory behaviour than the controls which was due to the significantly higher levels of lying/resting behaviour shown by these animals. This resting behaviour may have included sleeping but, as we didn't conduct sleep/EEG measurements, this is impossible to tell. The increased resting behaviour in the exercised animals was mainly displayed during the second half of the 30-min period indicating that this behaviour was most likely not instigated by a general lack of interest in the environment. The increased resting behaviour is also most likely not a result of increased tiredness because rats (and mice [20]) run hardly during the second half of the night. Rather, the increased resting behaviour appears to be a reflection of a decreased emotionality in the exercised rats, cf. the animal explores the cage after which it returns to its normal behaviour of that time of the day, being resting or sleeping. This reduced emotionality dovetails with the attenuated glucocorticoid response in these animals (see also below) and corresponds with the decreased anxiety-related behaviour we reported recently in voluntarily exercising mice [19].

One of the principal findings of this study is that exercised rats showed distinct stressor-specific responses in plasma corticosterone levels, but not in plasma ACTH levels. In view of the similar ACTH responses in exercised and control rats, it appears that the distinct glucocorticoid responses are not due to differential regulation at the hypothalamic-pituitary level. This notion is strengthened by the absence of changes in GR expression in the anterior pituitary and parvocellular PVN, and in CRH, AVP and oxytocin expression in the parvocellular

PVN. Brain regions such as the hippocampus and prefrontal cortex known to afferently control the activity of the parvocellular PVN [12, 15, 40, 45] showed a mixed picture of changes in corticosteroid receptor mRNA expression. After voluntary exercise, hippocampal MR mRNA levels were unchanged whereas GR mRNA levels showed increases in distinct subregions of the hippocampus. However, the ventral (temporal) part of the hippocampus, i.e. the part predominantly involved in HPA axis regulation [45], showed fewer changes in GR expression than the dorsal (septal) part, which is mostly engaged in the processing of cognitive information [46]. This distinction corresponds with the lack of changes in neuropeptide expression in the parvocellular PVN. Previously, it has been reported that negative feedback efficacy is normal in exercising rats [33]. In view of the unchanged stress-induced ACTH responses and the largely unchanged GR expression levels in the anterior pituitary and neuroendocrine-relevant brain structures, our data seem to concur with this report. Nevertheless, although unlikely, it cannot be completely excluded that differences in plasma ACTH after these stressors were missed, because only one post-stress sample was collected. Other researchers found in *forced* exercised rats blunted plasma ACTH and unchanged corticosterone responses to foot shock and forced swimming [47–49], underlining once more that forced exercise, in contrast to voluntary exercise, induces chronic stress-like changes in the HPA axis.

The changes in the plasma corticosterone responses to stress in the exercised rats were profoundly stressor-specific. The exercised animals produced a greatly increased glucocorticoid response to a physically demanding, potentially life-threatening challenge such as forced swimming whereas the response to the mild psychological stress of a novel environment was strongly attenuated. Previously, we reported that exercised mice show a virtually identical pattern of responses to physically demanding and mild psychological stress situations [17, 18], indicating that the stress physiological implications of exercise may be largely similar across species. In view of the largely unchanged hypothalamic-pituitary component of the HPA axis of exercised rats, it appears that modulation of the glucocorticoid responses to the different stressors is mainly regulated at the adrenal level presumably by local intra-adrenal mechanisms and/or changes in the activity of neural inputs to the adrenal gland. We found a significant increase in size of the left and right adrenal cortices of exercised rats suggesting an enhanced glucocorticoid secretory capacity in these animals. Further-

more, we showed that long-term voluntary exercise in rats indeed results in alterations in neural input to the adrenal gland because of the risen (right) adrenomedullary TH mRNA expression in these animals. Thus, exercised rats, similar to exercised mice [17, 18], show an increased secretory capacity of adrenaline in their, at least right adrenomedullary, chromaffin cells. Adrenaline has been shown to stimulate adrenal steroidogenesis and glucocorticoid secretion [50]. Adrenomedullary adrenaline secretion is under the control of splanchnic nerve input (including sympathetic nerve fibres) and a host of neuropeptides including enkephalins, vaso-active intestinal peptide (VIP), and neuropeptide Y [50]. The splanchnic adrenal innervation (which includes the sympathoadrenomedullary innervation) regulates adrenocortical sensitivity to ACTH stimulation, because sectioning the splanchnic nerve decreases the adrenal response to ACTH [51, 52] whereas stimulation augments it [53, 54]. Stressful stimuli are also known to activate the sympathetic nervous system resulting in rapid increases in circulating adrenaline, stemming mainly from chromaffin cells in the adrenal medulla, and noradrenalin, released largely from the neuro-sympathetic system [55–58]. Furthermore, and of relevance for the differential glucocorticoid responses to stress in the exercised rats, stressors that are physically demanding such as forced swimming evoke much larger increases in adrenaline than psychological challenges such as novel cage stress which are hardly physically demanding [59–62]. As mentioned, exercised animals show an enhanced adrenaline secretory capacity and indeed higher maximal adrenaline responses to various physically demanding challenges have been observed in exercised subjects [63–69]. Therefore, in aggregate it may be postulated that in exercised animals an enhanced adrenaline response upon a physically demanding challenge (e.g. forced swimming) may be responsible for the augmented glucocorticoid response in these animals. Reversely, an attenuated adrenaline response, despite enhanced adrenaline secretory capacity, may play a role in the observed decreased glucocorticoid response to novelty stress in the exercised rats. An attenuated sympathoadrenal response would correspond with the decreased anxiety levels presented by exercised animals in the elevated plus-maze and light-dark box tests [19] and in the novel environment paradigm in the present study. Therefore, we hypothesize that in exercised animals the glucocorticoid response to psychological challenges such as novelty is attenuated due to inhibition of sympathoadrenomedullary outflow involving changes in afferent sympatho-inhibitory and sympatho-excitatory projections,

local inhibitory mechanisms at the adrenal level and/or other so far unknown mechanisms.

Taken together these neuroendocrine observations show that exercised animals demonstrate improved control of glucocorticoid secretion during stressful conditions presenting stronger hormonal responses when high levels of glucocorticoid levels are physiologically required such as after physically demanding stressors and attenuated hormonal responses to psychological challenges when these animals have proven to be less anxious. The exact neurobiological mechanism underlying these effects of exercise is still unclear, but it has been shown that exercise brings patients relief of their anxiety and depressed mood, improves stress coping and increases vegetative stability [7–9] which we postulate is, at least in part, due to the often underestimated influence of the

sympathetic nervous system in modulating HPA axis function to maintain homeostasis. Importantly, the present study shows that the regular performance of exercise evokes similar sympathoadrenomedullary and HPA axis changes in rats and mice. Therefore, in view of the similarity of their stress physiological systems, our findings on the effects of exercise on the stress response and stress coping may be of significance for most mammals, including man.

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References

- 1 Friedman JE, Ferrara CM, Aulak KS, Hatzoglou M, McCune SA, Park S, Sherman WM: Exercise training down-regulates ob gene expression in the genetically obese SHHF/Mcc-fa(cp) rat. *Horm Metab Res* 1997;29:214–219.
- 2 Lambert GW, Jonsdottir IH: Influence of voluntary exercise on hypothalamic norepinephrine. *J Appl Physiol* 1998;85:962–966.
- 3 Kramer JM, Plowey ED, Beatty JA, Little HR, Waldrop TG: Hypothalamus, hypertension, and exercise. *Brain Res Bull* 2000;53:77–85.
- 4 Gielen S, Schuler G, Hambrecht R: Exercise training in coronary artery disease and coronary vasomotion. *Circulation* 2001;103:e1–e6.
- 5 Pedersen BK, Hoffman-Goetz L: Exercise and the immune system: regulation, integration, and adaptation. *Physiol Rev* 2000;80:1055–1081.
- 6 Jonsdottir IH: Neuropeptides and their interaction with exercise and immune function. *Immunol Cell Biol* 2000;78:562–570.
- 7 Steptoe A, Edwards S, Moses J, Mathews A: The effects of exercise training on mood and perceived coping ability in anxious adults from the general population. *J Psychosom Res* 1989;33:537–547.
- 8 Byrne A, Byrne DG: The effect of exercise on depression, anxiety and other mood states: a review. *J Psychosom Res* 1993;37:565–574.
- 9 Salmon P: Effects of physical exercise on anxiety, depression, and sensitivity to stress: a unifying theory. *Clin Psychol Rev* 2001;21:33–61.
- 10 Dimeo F, Bauer M, Varahram I, Proest G, Halter U: Benefits from aerobic exercise in patients with major depression: a pilot study. *Br J Sports Med* 2001;35:114–117.
- 11 Steckler T, Holsboer F, Reul JMHM: Glucocorticoids and depression; in Holly JMP (ed): *Baillière's Best Practice and Research. Stress and Immunity: The Neuroendocrine Link*. London, Baillière Tindall, 1999, pp 597–614.
- 12 Reul JMHM, Gesing A, Droste SK, Stec ISM, Weber A, Bachmann CG, Bilanz-Bleuel A, Holsboer F, Linthorst ACE: The brain mineralocorticoid receptor: greedy for ligand, mysterious in function. *Eur J Pharmacol* 2000;405:235–249.
- 13 Reul JMHM, Holsboer F: Corticotropin-releasing factor receptors 1 and 2 in anxiety and depression. *Curr Opin Pharmacol* 2002;2:23–33.
- 14 Dinan TG: Noradrenergic and serotonergic abnormalities in depression: stress-induced dysfunction? *J Clin Psychiatry* 1996;57(suppl 4):14–18.
- 15 De Kloet ER, Vreugdenhil E, Oitzl MS, Joëls M: Brain corticosteroid receptor balance in health and disease. *Endocr Rev* 1998;19:269–301.
- 16 Sapolsky RM, Romero LM, Munck AU: How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev* 2000;21:55–89.
- 17 Droste SK, Gesing A, Ulbricht S, Müller MB, Linthorst ACE, Reul JMHM: Effects of long-term voluntary exercise on the mouse hypothalamic-pituitary-adrenocortical axis. *Endocrinology* 2003;144:3012–3023.
- 18 Droste SK, Schweizer MC, Ulbricht S, Reul JMHM: Long-term voluntary exercise and the mouse hypothalamic-pituitary-adrenocortical axis: impact of concurrent treatment with the antidepressant drug tianeptine. *J Neuroendocrinol* 2006;18:915–925.
- 19 Binder E, Droste SK, Ohl F, Reul JMHM: Regular voluntary exercise reduces anxiety-related behaviour and impulsiveness in mice. *Behav Brain Res* 2004;155:197–206.
- 20 Lancel M, Droste SK, Sommer S, Reul JMHM: Influence of regular voluntary exercise on spontaneous and social stress-affected sleep in mice. *Eur J Neurosci* 2003;17:2171–2179.
- 21 Afonso VM, Eikelboom R: Relationship between wheel running, feeding, drinking, and body weight in male rats. *Physiol Behav* 2003;80:19–26.
- 22 Looy H, Eikelboom R: Wheel running, food intake, and body weight in male rats. *Physiol Behav* 1989;45:403–405.
- 23 Harri M, Lindblom J, Malinen H, Hyttinen M, Lapveteläinen T, Eskola S, Helminen HJ: Effect of access to a running wheel on behavior of C57BL/6J mice. *Lab Anim Sci* 1999;49:401–405.
- 24 Cooper JJ, Nicol CJ: Stereotypic behavior affects environmental preferences in bank voles, *Chlethronomys glareolus*. *Anim Behav* 1991;41:971–977.
- 25 Cooper JJ, Nicol CJ: Stereotypic behavior in wild caught and laboratory bred bank voles (*Clethrionomys glareolus*). *Anim Welfare* 1996;5:245–257.
- 26 Gesing A, Bilanz-Bleuel A, Droste SK, Linthorst ACE, Holsboer F, Reul JMHM: Psychological stress increases hippocampal mineralocorticoid receptor levels: involvement of corticotropin-releasing hormone. *J Neurosci* 2001;21:4822–4829.
- 27 Reul JMHM, Stec I, Söder M, Holsboer F: Chronic treatment of rats with the antidepressant amitriptyline attenuates the activity of the hypothalamic-pituitary-adrenocortical system. *Endocrinology* 1993;133:312–320.

- 28 Reul JM, Labeur MS, Grigoriadis DE, De Souza EB, Holsboer F: Hypothalamic-pituitary-adrenocortical axis changes in the rat after long-term treatment with the reversible monoamine oxidase-A inhibitor moclobemide. *Neuroendocrinology* 1994;60:509–519.
- 29 Simmons DM, Arriza JL, Swanson LW: A complete protocol for in situ hybridization of messenger RNAs in brain and other tissues with radio-labeled single-stranded RNA probes. *J Histotechnol* 1989;12:169–181.
- 30 Dallman MF, Akana SF, Cascio CS, Darlington DN, Jacobson L, Levin N: Regulation of ACTH secretion: variations on a theme of B. *Rec Prog Horm Res* 1987;43:113–173.
- 31 Hamelink C, Tjurmina O, Damadzic R, Young WS, Weihe E, Lee HW, Eiden LE: Pituitary adenylate cyclase-activating polypeptide is a sympathoadrenal neurotransmitter involved in catecholamine regulation and glucohomeostasis. *Proc Natl Acad Sci USA* 2002;99:461–466.
- 32 Engeland WC, Arnhold MM: Neural circuitry in the regulation of adrenal corticosterone rhythmicity. *Endocrine* 2005;28:325–332.
- 33 Fediuc S, Campbell JE, Riddell MC: Effect of voluntary wheel running on circadian corticosterone release and on HPA axis responsiveness to restraint stress in Sprague-Dawley rats. *J Appl Physiol* 2006;100:1867–1875.
- 34 Enevoldsen LH, Stallknecht B, Fluckey JD, Galbo H: Effect of exercise training on in vivo lipolysis in intra-abdominal adipose tissue in rats. *Am J Physiol Endocrinol Metab* 2000;279:E585–E592.
- 35 Leshner AI: The adrenals and the regulatory nature of running wheel activity. *Physiol Behav* 1971;6:551–558.
- 36 Allen DL, Harrison BC, Maass A, Bell ML, Byrnes WC, Leinwand LA: Cardiac and skeletal muscle adaptations to voluntary wheel running in the mouse. *J Appl Physiol* 2001;90:1900–1908.
- 37 Ingle DJ: The time for the occurrence of cortico-adrenal hypertrophy in rats during continued work. *Am J Physiol* 1938;124:627–630.
- 38 Kjaer M: Regulation of hormonal and metabolic responses during exercise in humans. *Exerc Sport Sci Rev* 1992;20:161–184.
- 39 Riss W, Burstein SD, Johnson RW, Lutz A: Morphologic correlates of endocrine and running activity. *J Comp Physiol Psychol* 1959;52:618–620.
- 40 Reul JM, Droste SK: The hypothalamic-pituitary-adrenal axis as a dynamically organized system: lessons from exercising mice; in Steckler T, Kalin NH, Reul JM (eds): *Handbook of Stress and the Brain. Part 1. The Neurobiology of Stress. Techniques in Behavioral and Neural Sciences*. Amsterdam, Elsevier, 2005, vol 15, pp 95–112.
- 41 Kjaer M: Adrenal medulla and exercise training. *Eur J Appl Physiol* 1998;77:195–199.
- 42 Stallknecht B, Kjaer M, Ploug T, Maroun L, Ohkuwa T, Vinten J, Mikines KJ, Galbo H: Diminished epinephrine response to hypoglycemia despite enlarged adrenal medulla in trained rats. *Am J Physiol* 1990;259:R998–R1003.
- 43 Schmidt KN, Gosselin LE, Stanley WC: Endurance exercise training causes adrenal medullary hypertrophy in young and old Fischer 344 rats. *Horm Metab Res* 1992;24:511–515.
- 44 Bedford TG, Tipton CM, Wilson NC, Oppinger RA, Gisolfi CV: Maximal oxygen consumption of rats and its changes with various experimental procedures. *J Appl Physiol* 1979;47:1278–1283.
- 45 Herman JP, Figueiredo H, Mueller NK, Ulrich-Lai Y, Ostrander MM, Choi DC, Cullinan WE: Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. *Front Neuroendocrinol* 2003;24:151–180.
- 46 Moser MB, Moser EI: Functional differentiation in the hippocampus. *Hippocampus* 1998;8:608–619.
- 47 Dishman RK, Bunnell BN, Youngstedt SD, Yoo HS, Mougey EH, Meyerhoff JL: Activity wheel running blunts increased plasma adrenocorticotrophin (ACTH) after footshock and cage-switch stress. *Physiol Behav* 1998;63:911–917.
- 48 Watanabe T, Morimoto A, Sakata Y, Tan N, Morimoto K, Murakami N: Running training attenuates the ACTH responses in rats to swimming and cage-switch stress. *J Appl Physiol* 1992;73:2452–2456.
- 49 Watanabe T, Morimoto A, Sakata Y, Wada M, Murakami N: The effect of chronic exercise on the pituitary-adrenocortical response in conscious rats. *J Physiol* 1991;439:691–699.
- 50 Ehrhart-Bornstein M, Hinson JP, Bornstein SR, Scherbaum WA, Vinson GP: Intraadrenal interactions in the regulation of adrenocortical steroidogenesis. *Endocr Rev* 1998;19:101–143.
- 51 Edwards AV, Jones CT, Bloom SR: Reduced adrenal cortical sensitivity to ACTH in lambs with cut splanchnic nerves. *J Endocrinol* 1986;110:81–85.
- 52 Edwards AV, Jones CT: The effect of splanchnic nerve section on the sensitivity of the adrenal cortex to adrenocorticotrophin in the calf. *J Physiol* 1987;390:23–31.
- 53 Edwards AV, Jones CT: The effect of splanchnic nerve stimulation on adrenocortical activity in conscious calves. *J Physiol* 1987;382:385–396.
- 54 Engeland WC, Gann DS: Splanchnic nerve stimulation modulates steroid secretion in hypophysectomized dogs. *Neuroendocrinology* 1989;50:124–131.
- 55 Goldstein DS, McCarty R, Polinsky RJ, Kopin JJ: Relationship between plasma norepinephrine and sympathetic neural activity. *Hypertension* 1983;5:552–559.
- 56 Kvetnansky R, Nemeth S, Vigas M, Oprsalova Z, Juricova J: Plasma catecholamines in rats during adaptation to intermittent exposure to different stressors; in Usdin E, Kvetnansky R, Axelrod J (eds): *Stress: The Role of Catecholamines and Other Neurotransmitters*. New York, Gordon & Breach, 1984, pp 537–562.
- 57 McCarty R: Stress, behavior and experimental hypertension. *Neurosci Biobehav Rev* 1983;7:493–502.
- 58 Micalizzi ER, Pals DT: Evaluation of plasma norepinephrine as an index of sympathetic neuron function in the conscious, unrestrained rat. *Life Sci* 1979;24:2071–2076.
- 59 De Boer SF, Slangen JL, van der Gugten J: Adaptation of plasma catecholamine and corticosterone responses to short-term repeated noise stress in rats. *Physiol Behav* 1988;44:273–280.
- 60 De Boer SF, Koopmans SJ, Slangen JL, van der Gugten J: Effects of fasting on plasma catecholamine, corticosterone and glucose concentrations under basal and stress conditions in individual rats. *Physiol Behav* 1989;45:989–994.
- 61 De Boer SF, Koopmans SJ, Slangen JL, van der Gugten J: Plasma catecholamine, corticosterone and glucose responses to repeated stress in rats: effect of interstressor interval length. *Physiol Behav* 1990;47:1117–1124.
- 62 Scheurink AJ, Steffens AB, Bouritius H, Dreteler GH, Bruntink R, Remie R, Zaagsma J: Adrenal and sympathetic catecholamines in exercising rats. *Am J Physiol* 1989;256:R155–R160.
- 63 Alho H, Koistinaho J, Kovanen V, Suominen H, Hervonen A: Effect of prolonged physical training on the histochemically demonstrable catecholamines in the sympathetic neurons, the adrenal gland and extra-adrenal catecholamine storing cells of the rat. *J Auton Nerv Syst* 1984;10:181–191.
- 64 Kjaer M, Mikines KJ, Christensen NJ, Tronier B, Vinten J, Sonne B, Richter EA, Galbo H: Glucose turnover and hormonal changes during insulin-induced hypoglycemia in trained humans. *J Appl Physiol* 1984;57:21–27.
- 65 Kjaer M, Christensen NJ, Sonne B, Richter EA, Galbo H: Effect of exercise on epinephrine turnover in trained and untrained male subjects. *J Appl Physiol* 1985;59:1061–1067.
- 66 Kjaer M, Farrell PA, Christensen NJ, Galbo H: Increased epinephrine response and inaccurate glucoregulation in exercising athletes. *J Appl Physiol* 1986;61:1693–1700.
- 67 Kjaer M, Bangsbo J, Lortie G, Galbo H: Hormonal response to exercise in humans: influence of hypoxia and physical training. *Am J Physiol* 1988;254:R197–R203.
- 68 Kjaer M, Galbo H: Effect of physical training on the capacity to secrete epinephrine. *J Appl Physiol* 1988;64:11–16.
- 69 Leblanc J, Jobin M, Cote J, Samson P, Labrie A: Enhanced metabolic response to caffeine in exercise-trained human subjects. *J Appl Physiol* 1985;59:832–837.