Forced swim test

In this test, mice were forced to swim for 5 min inside a narrow plexiglass cylinder filled with water of 25 °C. Three behaviours were recorded in this test: immobility (floating in the water without struggling), swimming (making active swimming motions), and climbing (making active movements with the forepaws directed against the wall).

This test has been used as an acute psychological stressor to investigate differences between LAL and SAL mice in HPA reactivity (chapter 2) and in stress-induced suppression of hippocampal cell proliferation rate (chapter 5). This test was also used to measure the effect of chronic psychosocial stress on forced swimming behaviour in LAL and SAL mice (chapter 3). Finally, this test was used to measure the effects of two 5-HT1a receptor agonists on forced swimming behaviour in LAL and SAL mice (chapter 6).
Chapter 2

DIFFERENCES IN BASAL AND STRESS-INDUCED HPA REGULATION OF WILD HOUSE MICE SELECTED FOR HIGH AND LOW AGGRESSION

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This paper is a tribute to Béla Bohus, whose untimely death is a great loss to us all
Abstract

Male wild house-mice, selected for short (SAL) and long (LAL) attack latency, show distinctly different behavioural strategies in coping with environmental challenges. In this study, we tested the hypothesis that this difference in coping style is associated with a differential stress responsiveness of the hypothalamic-pituitary-adrenal (HPA) system. SAL rather than LAL mice showed a clear fluctuation in circulating corticosterone concentrations around the circadian peak with significantly higher levels in the late light phase. LAL mice showed lower basal ACTH levels and higher thymic and spleen weights compared to SAL mice. Under basal conditions, glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) mRNA in the hippocampus and corticotropin-releasing hormone (CRH) mRNA in the paraventricular nucleus of the hypothalamus were not different between the two lines. Forced swimming for 5 min induced high immobility behaviour in LAL mice which was associated with an enhanced and prolonged corticosterone response compared to SAL mice, while absolute ACTH levels did not differ. In addition, LAL mice showed an increase in hippocampal MR mRNA (but not GR) and hypothalamic CRH mRNA at 24 h after forced swimming. In conclusion, a genetic trait in coping style of wild house-mice is associated with an idiosyncratic pattern of HPA activity, and greater responsiveness of physiological and molecular stress markers in LAL mice. In view of the profound differences in behavioural traits and stress system reactivity, these mouse lines genetically selected for attack latency present an interesting model for studying the mechanism underlying individual variation in susceptibility to stress-related psychopathology.
Introduction

Several studies on individual vulnerability to stress-related disease are based on the use of lines generated by genetic selection on behavioural differences (Korte et al., 1997; Schouten and Wiegant, 1997; Carere et al., 2001). These selection lines are thought to represent the extremes of behavioural response patterns that coexist in a mammalian population (Henry and Stephens, 1977). One general response pattern is characterized by territorial control and aggression, whereas the other is characterized by immobility and low levels of aggression (Koolhaas et al., 1999).

We have used lines of wild house-mice selected on the basis of attack latency. Subsequent findings suggest that this difference in attack latency is part of a more general difference in behavioural strategy towards environmental challenges. SAL (Short Attack Latency; high aggressive) mice are characterized by the ‘active’ coping style whereas LAL (Long Attack Latency, low to non aggressive) mice show the ‘passive’ coping style (Benus et al., 1989, 1991a,b; Sluyter et al., 1996). Recently, the plasma corticosterone concentration during the early dark phase was found to be higher in LAL mice than in SAL mice (Korte et al., 1996). In rats, chicks and pigs, coping style was found to be associated with hypothalamic-pituitary-adrenal (HPA) activity (Korte et al., 1997; De Boer et al., 1990a,b; Schouten and Wiegant, 1997). This suggests that differences in coping style are not only reflected by differences in behavioural performance but also by differential physiological and neuroendocrine responses.

In this study we test the hypothesis that SAL and LAL mice differ in the stress responsiveness of the HPA system. For this purpose, corticosterone and ACTH levels were measured under basal (home cage) and stress (forced swimming) conditions. In addition, glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) mRNA in the hippocampus and corticotropin-releasing hormone (CRH) mRNA in the paraventricular nucleus (PVN) of the hypothalamus were measured under basal conditions and after exposure to forced swimming. The data suggest an altered regulation of the HPA system under basal conditions and greater responsiveness of physiological and molecular stress markers in LAL mice.

Material and methods

Animals

Two mouse lines, genetically selected for attack latency, originated from a colony of wild house-mice (Mus musculus domesticus) maintained at the University of Groningen, The Netherlands, since 1971. The mice were housed in Plexiglas cages (17 x 11 x 13 cm) in a room with an artificial 12:12 light/dark cycle (lights on from 0030 to 1230). Standard laboratory chow and water was available ad libitum. The mice were weaned at 3-4 weeks of age, and were paired male-female at the age of 6-8 weeks. For these experiments, male
mice were used at the age of 17 weeks (+/- 2 weeks). The SAL males came from the 62-
63rd generation of selection, the LAL males from the 36-38th generation. Only SAL mice
with an attack latency of less than 50 s and only non-attacking LAL mice were used for the
experiments. The attack latency test has been extensively described by Van Oortmerssen
and Bakker (1981). Briefly, genetically selected SAL and LAL mice at the age of 92-100
days are confronted with a standard non-aggressive opponent in a neutral cage. The time it
takes before a SAL or LAL mouse attacks the non-aggressive opponent is measured on
three consecutive days. The attack latency score is the mean of these daily scores. Neither
SAL nor LAL mice experienced a social defeat. All experiments were in accordance with
the regulations of the Committee for Use of Experimental Animals of the University of
Groningen (DEC nr. 2326).

Experimental procedures
To measure basal corticosterone and ACTH levels in blood, mice were housed under
basal conditions (home cage) and were decapitated under CO_2 anaesthesia at two time
points in the late light phase (0900 and 1200, n = 8 of each line per time point) and at two
time points in the early dark phase (1500 and 1800, n = 8 of each line per time point).
Trunk blood was collected for corticosterone and ACTH measurements. From 18 SAL
mice and 19 LAL mice, body weight was measured and organs were removed and weighed.

HPA reactivity was measured by exposing mice to forced swimming. Mice were
forced to swim in a narrow Plexiglas cylinder (diameter of 10 cm) filled with water of 25°C
for 5 min before they returned to their home cage. This procedure was a modified version
of the test described by Porsolt et al. (1977). The following three behaviours were recorded
using The Observer, version 3.0 (Noldus Information Technology, Wageningen, The
Netherlands) to determine a line difference in coping with the stressor: immobility (floating
in the water without struggling, making only those movements necessary to keep the head
above the water), swimming (making active swimming motions and moving around in the
cylinder) and climbing (making active movements with the forepaws in and out the water,
usually directed against the wall). All experiments were performed between 1100 and 1200.
For corticosterone and ACTH measurements, five to eight mice of each line were killed 15
(t = 15) or 90 (t = 90) min later by decapitation under CO_2 anaesthesia, and trunk blood was
collected. Stress-induced levels of corticosterone and ACTH were compared to basal
hormone levels obtained from SAL and LAL mice (n = 8 per line) that were housed in a
home cage and decapitated at 1200. To measure MR, GR and CRH mRNA expression, 7
mice of each line were subjected to forced swimming and 24 h later the brains were rapidly
removed, quickly frozen in ice-cold p-heptane and stored in –80°C for subsequent in situ
hybridisation. Control SAL and LAL mice (n = 8 per line) were housed under basal
conditions and were decapitated in the same time period.

Radioimmunoassay for corticosterone and ACTH
Trunk blood was collected in chilled tubes containing EDTA for determination of
corticosterone and ACTH levels. Blood samples were centrifuged at 2600g for 10 min at
4°C. Plasma samples were stored at –20°C until assayed. Plasma corticosterone was
determined in duplo using a commercially available radioimmunoassay (Mouse
Corticosterone RIA Kit, ICN Biomedicals, Costa Mesa, CA). The detection limit of the
assay was 3 ng corticosterone/ml with an intra-assay variance of 4.4% and inter-assay
variance 6.5%. A double-antibody radioimmunoassay (ACTH RIA kit, Nichols Institute
Diagnostics, San Juan Capistrano, CA) with intra-assay and inter-assay variances of 3.2% and 7.8% was used to measure plasma ACTH. The detection limit of the assay was 1 pg ACTH/ml.

**In situ hybridisation**

Brain tissue sections of 20 µm were cut on a cryostat and thaw-mounted on poly-L-lysine-coated slides. These slides were stored at –80°C until the time of hybridisation. The hybridisation protocol was adopted from Meijer et al. (1997), with some minor modifications. Briefly, prior to hybridisation the sections were fixed in 4% paraformaldehyde, permeabilized by proteinase K treatment, acetylated twice with 0.25 % acetic anhydride in 0.1 M triethanolamine and dehydrated in a graded ethanol series. Riboprobes were generated from linearized constructs containing the respective cDNA’s in pBluescript. A 500 bp SalI-HindIII fragment of exon 2 of the mouse gene was used for GR and a 1.2 kb Ncol-EcoRI fragment of the mouse MR exon 2 for the MR (courtesy of Dr. T. Cole). The cRNA from CRH was transcribed from a 1-kb cDNA insert in pGEM 4 containing full-length coding region of rat CRH. 35S-UTP-labeled antisense probes were generated using the appropriate polymerase using a standard protocol. A hybridisation mix was prepared containing 60% deionised formamide, 10% Dextran SO4, 2x SSC, 0.1 mg/ml yeast tRNA, 0.1 mg/ml sssDNA, 10 mM DTT, 0.05 M PBS. All radiolabelled probes were diluted to 20 x 10^6 dpm/ml. 100 µl of these mixtures was applied to each slide, which was then covered with a coverslip. The sections were hybridized overnight in a moisturized chamber at 55 °C. The next day, coverslips were removed carefully and sections were washed in 2x SSC for 10 min at room temperature. After washing, sections were treated with RNase (2 mg/100 ml in 0.5 M NaCl, 0.1 M Tris, pH 7.5) at 37 °C for 10 min and subsequently washed at 55 °C in 2x SSC for 10 min, 1x SSC for 10 min, 0.1x SSC for 2x 30 min and finally at room temperature in 0.1x SSC for 5 min. Sections were dehydrated in an ethanol series (70%, 80%, 96% and 100% ethanol) and dried on the air. Hybridized slices were exposed to a X-Omat AR film (Kodak, Rochester, NY) for 3 weeks. Optical density was determined by using an automatic image analysis system (Quantimet 500, Leica, Cambridge). For GR and MR mRNA expression, the optical density of CA1, dentate gyrus (GR and MR), CA2 and CA3 (MR) were determined in three hippocampal sections of each animal. The optical density of a small area between the CA1 and dentate gyrus was used for tissue background. To determine CRH mRNA expression, the optical density of one or two sections of each animal containing the PVN were measured. A nonhybridized region outside the PVN was measured for tissue background.

**Statistical analysis**

Analysis of variance (ANOVA) was used to analyse line and time differences in basal levels of corticosterone and ACTH. Body weight and relative weights of various organs were analysed by an unpaired Student’s t-test. Behaviour in the forced swimming test was analysed by a multivariate ANOVA (MANOVA). To determine line, stress, and interaction effects for corticosterone and ACTH responses to forced swimming, ANOVA was used. The mRNA expression of MR, GR and CRH was analysed by MANOVA (GR, MR) or ANOVA (CRH) to determine line, stress, and interaction effects. When a significance was revealed, appropriate pairwise comparisons (LSD test) were done based on the estimated marginal means. For all tests the software package SPSS (version 9) was used. Data are presented as mean ± S.E.M. Significance was taken at P < 0.05.
Results

Basal release of corticosterone and ACTH

Plasma corticosterone and ACTH levels were measured by decapitation at two time points in the late light phase (0900 and 1200) and at two time points in the early dark phase (1500 and 1800). No effect of line, time, or interaction was observed for corticosterone (Fig. 1A), while a line difference was found for ACTH ($F_{(1,54)} = 21.185, P < 0.001$) (Fig. 1C). To reveal a possible time difference between the light and dark period, the two time points in the light phase and the two time points in the dark phase were pooled. Under these conditions, an interaction effect between line and time of the day was found for corticosterone levels ($F_{(1,58)} = 6.074, P < 0.05$). Plasma corticosterone levels of SAL mice were significantly higher in the light period compared to LAL mice ($P < 0.05$) and compared to the dark period ($P < 0.001$) (Fig. 1B). Corticosterone levels of LAL mice did not show a fluctuation over the light and dark period (Fig. 1B). A significant line difference was found for basal plasma ACTH levels ($F_{(1,58)} = 21.356, P < 0.001$), which were significantly lower in LAL mice compared to SAL mice in the light ($P < 0.005$) and dark period ($P < 0.01$) (Fig. 1D). No time effect of ACTH levels was observed in either line (Fig. 1D).

Body weight and organ weights

Several organs were weighed as possible peripheral indicators of an altered HPA functioning. The mouse lines showed no difference in body weight nor in the relative weights of adrenals, seminal vesicles and testes. Relative thymic weights ($P < 0.001$) and spleen weights ($P < 0.005$) were found to be significantly higher in LAL mice than in SAL mice (Table 1).

Table 1. Absolute body weight (g) and relative organ weights (mg/g body weight) of SAL ($n = 18$) and LAL ($n = 19$) mice. Sem Ves, seminal vesicles.

<table>
<thead>
<tr>
<th></th>
<th>Body weight</th>
<th>Adrenals</th>
<th>Thymus</th>
<th>Spleen</th>
<th>Sem Ves</th>
<th>Testes</th>
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<tr>
<td>SAL</td>
<td>20.44 ± 0.45</td>
<td>0.16 ± 0.004</td>
<td>1.09 ± 0.06</td>
<td>1.81 ± 0.06</td>
<td>4.96 ± 0.14</td>
<td>8.38 ± 0.19</td>
</tr>
<tr>
<td>LAL</td>
<td>20.92 ± 0.57</td>
<td>0.16 ± 0.006</td>
<td>1.39 ± 0.04*</td>
<td>2.14 ± 0.09**</td>
<td>5.20 ± 0.28</td>
<td>8.56 ± 0.26</td>
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* $P < 0.001$, ** $P < 0.005$ vs. SAL mice, Student’s $t$-test.

Behaviour during forced swimming

Analysis of behaviour of SAL and LAL mice during forced swimming for 5 min revealed a significant line difference ($F_{(1,25)} = 10.532, P < 0.001$). LAL mice showed significantly more immobility behaviour ($P < 0.001$) and less climbing ($P = 0.001$) and swimming ($P < 0.05$) behaviour than SAL mice (Table 2).
Fig. 1. Plasma corticosterone and ACTH levels were measured in the late light phase (0900 and 1200) and in the early dark phase (1500 and 1800) in SAL and LAL mice (A and C). In (B) and (D), the time points within each phase were pooled, to reveal a possible time difference between the light and dark period. (A) No significant line, time, or interaction effect was found for corticosterone levels. (B) SAL mice showed significantly higher corticosterone levels in the late light phase compared to LAL mice and compared to the early dark phase. (C) ACTH levels were significantly different between SAL and LAL mice at 1200 and 1500. (D) LAL mice showed significantly lower ACTH levels than SAL mice in the light and in the dark period. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, ANOVA.
**Table 2.** Behaviour of SAL ($n = 14$) and LAL ($n = 13$) mice in the forced swim test (5 min)

<table>
<thead>
<tr>
<th></th>
<th>Climbing</th>
<th>Swimming</th>
<th>Immobility</th>
</tr>
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<tbody>
<tr>
<td>SAL</td>
<td>$27.7 \pm 4.5$</td>
<td>$28.2 \pm 2.7$</td>
<td>$44.7 \pm 4.0$</td>
</tr>
<tr>
<td>LAL</td>
<td>$9.0 \pm 1.5^*$</td>
<td>$19.1 \pm 2.8^{**}$</td>
<td>$73.3 \pm 3.0^*$</td>
</tr>
</tbody>
</table>

$^* P \leq 0.001$, $^* P < 0.05$ vs. SAL mice, MANOVA.

**Effect of the swim stressor on corticosterone and ACTH levels**

In order to assess line differences in the stress-induced response of corticosterone and ACTH, mice were decapitated at $t = 15$ or $t = 90$ min after 5 min of forced swimming (Fig. 2). Stress-induced levels of corticosterone and ACTH were compared to basal hormone levels obtained from SAL and LAL mice decapitated at 1200 and used in the experiment presented in Fig. 1.

Forced swimming induced a significant line ($F_{(1,35)} = 11.590, P < 0.005$), time ($F_{(2,35)} = 61.677, P < 0.001$) and interaction ($F_{(2,35)} = 8.368, P < 0.005$) effect for corticosterone levels (Fig. 2A). Significantly higher stress-induced corticosterone levels were observed compared to basal levels at $t = 15$ in both lines ($P < 0.001$) and at $t = 90$ in LAL mice ($P < 0.05$). Corticosterone levels showed a significant decrease at $t = 90$ compared to $t = 15$ in both lines ($P < 0.001$). The stress-induced increase in absolute levels of corticosterone was significantly higher in LAL mice than in SAL mice at both time points ($t = 15: P < 0.005$, $t = 90: P < 0.05$). By expressing the stress-induced corticosterone levels as relative to basal levels (Fig. 2C), similar line ($F_{(1,22)} = 44.795, P < 0.001$), time ($F_{(1,22)} = 64.925, P < 0.001$) and interaction ($F_{(1,22)} = 5.832, P < 0.05$) effects were observed compared to absolute stress-induced corticosterone levels. Relative stress-induced corticosterone levels were also significantly higher in LAL mice than in SAL mice at both time points ($P < 0.001$ at $t = 15$ and $P < 0.005$ at $t = 90$).

Significant line ($F_{(1,35)} = 7.588, P < 0.01$) and time ($F_{(2,35)} = 8.927, P < 0.005$) effects were found for stress-induced ACTH levels (Fig. 2B). Absolute levels of ACTH were significantly increased at $t = 15$ after forced swimming compared to basal levels in SAL mice ($P < 0.005$) and LAL mice ($P < 0.05$). A decrease in ACTH levels was observed at $t = 90$ compared to $t = 15$ in both lines ($P < 0.01$ for SAL mice and $P < 0.05$ for LAL mice). When stress-induced ACTH levels were expressed as relative to basal ACTH levels, again a line ($F_{(1,22)} = 5.464, P < 0.05$) and time ($F_{(1,22)} = 5.797, P < 0.05$) effect was observed (Fig. 2D). In contrast to absolute levels, relative stress-induced levels of ACTH were higher in LAL mice than in SAL mice at $t = 15$ ($P < 0.05$).
Fig. 2. Plasma corticosterone and ACTH levels of SAL and LAL mice under basal conditions (home cage) and 15 (t = 15) and 90 (t = 90) min after 5 min of forced swimming at the end of the light phase. (A) Forced swimming induced a significant increase in corticosterone levels compared to basal levels at t = 15 in SAL and LAL mice and at t = 90 in LAL mice. At t = 90, corticosterone levels were significantly decreased compared to t = 15 in both lines. Corticosterone levels were significantly higher in LAL mice than in SAL mice at t = 15 and t = 90. (B) Forced swimming induced a significant increase in absolute levels of ACTH at t = 15 in both lines compared to their basal ACTH levels. At t = 90, ACTH levels were significantly decreased compared to t = 15 in both lines. Absolute ACTH levels were not significantly different between SAL and LAL mice. (C) The stress-induced relative increase in corticosterone (expressed as percentage from basal corticosterone levels) was higher in LAL mice than in SAL mice at both time points. (D) The stress-induced relative increase in ACTH (expressed as percentage from basal ACTH levels) showed a significantly higher level of ACTH at t = 15 in LAL mice than in SAL mice. a P at least < 0.05 vs. basal, b P at least < 0.05 vs. 15', * P < 0.05 vs. SAL, ** P < 0.005 vs. SAL, *** P < 0.001 vs. SAL, ANOVA.
Fig. 3. MR (A + B) and GR (C) mRNA expression in the hippocampus and CRH (D) mRNA expression in the paraventricular nucleus (PVN) of the hypothalamus of SAL and LAL mice under basal conditions (home cage) and 24 h after swim stress. No line difference was found in MR and GR mRNA expression under basal and stress conditions, except for the CA2 region (B), where levels of MR mRNA expression after stress were significantly higher in LAL than in SAL mice. Within the LAL line, MR mRNA expression showed a significant increase in all hippocampal regions 24 h after swim stress compared to basal (A+B). SAL mice showed only a significant increase in MR mRNA expression after swim stress in the CA1 region (A). GR mRNA expression was not affected by swim stress in neither the SAL nor the LAL mice (C). CRH mRNA expressed in the PVN did not differ between SAL and LAL under basal conditions, but was significantly increased in LAL mice after swim stress compared to SAL mice and LAL basal (D). * $P < 0.05$; ** $P \leq 0.005$, MANOVA (MR, GR) or ANOVA (CRH).
MR, GR and CRH mRNA expression under basal conditions and after acute stress

The mRNA expression of the two corticosteroid receptors, the MR and GR, and of CRH were measured under basal conditions and 24 h after exposure to forced swimming.

There was no line difference in MR and GR mRNA in the hippocampus of SAL and LAL mice under basal conditions. However, 24 h post-stress, the MR mRNA expression was significantly higher compared to basal conditions ($F_{(1,25)} = 6.899, P < 0.005$), reaching significance in SAL mice only in the CA1 region ($P < 0.05$), whereas in LAL mice, swim stress induced a significant increase in all hippocampal regions compared to basal (CA1, CA2, DG: $P < 0.005$; CA3: $P < 0.05$) (Fig. 3A+B). The swim stressor induced a small but significant higher expression of MR mRNA in the hippocampal CA2 region of LAL mice compared to SAL mice ($P < 0.05$). GR mRNA expression was not affected by forced swimming in either the SAL or the LAL line (Fig. 3C).

An interaction effect between line and stress was observed for CRH mRNA expression in the PVN ($F_{(1,23)} = 8.581, P < 0.01$). CRH mRNA expression, at 24 h after the swim stressor, was significant higher in LAL mice compared to SAL mice ($P = 0.005$) and compared to basal LAL mice ($P < 0.05$) (Fig. 3D).

Discussion

The present study was designed in order to test the hypothesis that wild house-mice, selected for short (SAL) and long (LAL) attack latency and displaying distinctly different coping styles, differ in stress responsiveness of the HPA axis. We report here, that LAL mice showed no variation in basal plasma corticosterone concentration around the circadian peak, while their basal ACTH levels were much lower than those in SAL mice. In response to a forced swim stressor, LAL mice showed an enhanced and prolonged corticosterone secretion, whereas ACTH levels were not different from those in SAL mice. In LAL rather than SAL mice, hippocampal MR and hypothalamic CRH mRNA expression were still enhanced at 24 h after exposure to the swim stressor. Collectively, the data suggest a profound line difference in HPA regulation under basal and acute stress conditions. A prominent outcome of the study is the apparent enhanced adrenocortical function displayed by individuals of the LAL line.

Basal levels of corticosterone and ACTH were measured at specific time points during the late light phase and early dark phase. Corticosterone levels were found to be higher in SAL mice at the end of the light phase (only when the two time points were pooled) than in LAL mice, whereas no line difference was observed at the beginning of the dark phase. The previously reported higher corticosterone values in LAL mice during the early dark phase (Korte et al., 1996) were not found but may be present as a trend. The observed low levels of ACTH in LAL mice,
indicate that the method described here was fast enough to accurately measure basal levels of HPA hormones.

The line difference in basal HPA activity around the circadian peak revealed two hallmarks. First, SAL but not LAL mice showed a fluctuation of corticosterone levels around the circadian peak. Although obviously more data are needed that include the circadian trough, the lack of day-night variation in corticosterone of LAL mice indicates at least a less pronounced circadian rhythm, which is also found to be a prominent feature in patients suffering from stress-related disorders such as, e.g., major depression (Weber et al., 2000). Second, basal ACTH levels were much lower in LAL mice than in SAL mice. Since a relatively small line difference in basal corticosterone output was observed only in the late light phase, this finding suggests enhanced adrenocortical sensitivity to ACTH in LAL mice. Several mechanisms may explain this presumed difference in ACTH sensitivity. One possibility is increased splanchnic innervation of the adrenal and thereby potentiating the steroidogenic response to ACTH (Edwards and Jones, 1993). Another possibility is the existence of an ACTH-independent mechanism that may involve adrenomedullary peptidergic systems. For instance, vasoactive intestinal peptide (VIP) released from the adrenal medulla following splanchnic nerve stimulation was shown to stimulate corticosterone secretion directly at the level of the adrenal gland (Bodnar et al., 1997). Thus, the corticosterone output of LAL mice seems more sensitive to ACTH, but shows less day-night variation.

Forced swimming for 5 min was used as an acute stressor to measure the stress responsiveness of the HPA system in the two mouse lines. There was a clear line difference in behaviour during forced swimming. LAL mice showed more immobility behaviour than SAL mice. These results support earlier findings in these mice and are consistent with the idea that mice genetically selected for aggression differ more generally in coping with environmental challenges (Benus et al., 1989, 1991a,b; Sluyter et al., 1996). This difference in coping style was found to be associated with a difference in stress responsiveness of the HPA axis. LAL mice showed a higher stress-induced increase in circulating corticosterone concentrations than SAL mice. Other studies also have shown that in general a passive coping style is associated with higher corticosterone output (De Boer et al., 1990b; Korte et al., 1997; Schouten and Wiegant, 1997). However, no direct correlation could be found between the behaviour in the forced swimming test and the hormonal responses. Absolute levels of stress-induced ACTH were not significantly different between the two mouse lines. This finding is consistent with the enhanced adrenocortical sensitivity for ACTH in LAL mice under basal conditions. The observation of a prolonged stress-induced increase in corticosterone levels in LAL mice reveals an impaired termination of the stress response. Whether this is solely due to increased adrenocortical sensitivity to ACTH or reveals additional alterations at other sites within the HPA axis (e.g., glucocorticoid feedback resistance) is not clear yet. The observed higher relative thymus and spleen weights in LAL mice suggest at least that the latter is possible.
Under basal conditions, plasma levels of ACTH were lower in LAL mice but CRH mRNA in the PVN did not show a line difference. This may indicate a decreased sensitivity for CRH at the pituitary level or for one of its co-secretagogues (e.g., vasopressin) in LAL mice. Alternatively, a different translation efficiency may open the possibility that there still can be a line difference in CRH bioavailability. Yet, acute swim stress induced a significant rise at 24 h post-stress in CRH mRNA in the PVN of solely the LAL mice, but this rise did not result in elevated basal ACTH or corticosterone levels at this time (Veenema, unpublished observation).

Effects of the 5-min swim stressor were also observed on the corticosteroid receptor system in hippocampus. In agreement with Van Riel et al. (2002) there were no line differences in basal MR and GR mRNA expression, and also there were no effects at 24 h post-stress in GR mRNA. This however, does not exclude possible changes in the expression of GR mRNA within the 24-h period. In contrast, a marked stress-induced rise in MR mRNA was observed in all hippocampal subfields in LAL mice, while this rise occurred in SAL mice only in the CA1 region. This finding agrees with the increased MR immunoreactivity observed by Gesing et al. (2001) at 24 h following exposure of rats to forced swimming and novelty, which appeared to be mediated by CRH. As LAL mice showed a marked stress-induced increase in MR mRNA, the observed increase in CRH mRNA in the PVN may have produced increased levels of CRH and subsequently elevated MR expression. Hippocampal MRs have been implicated in the control of behavioural reactivity (Ortizl et al., 1994) and the threshold or sensitivity of the stress response system (Ratka et al., 1989; De Kloet et al., 1998). This opens the possibility that the lines characterized by different coping styles may develop a distinctly different neuroendocrine and behavioural phenotype after chronic exposure to uncontrollable stress.

In conclusion, this study shows that a genetic trait in coping style in wild house-mice is associated with an idiosyncratic pattern of HPA activity. The SAL and LAL mice differed in the set point of HPA regulation with the LAL mice showing relatively reduced ACTH release and signs of adrenocortical hyperresponsiveness. In view of the profound differences in stress reactivity, behavioural traits, and critical neurotransmitter systems like the 5-HT system (Korte et al., 1996; Van Riel et al., 2002), the mouse lines genetically selected for attack latency present an interesting animal model to study the mechanisms underlying individual variation in susceptibility for stress-related psychopathology. Future investigations will focus on the biological consequences of differences in HPA function under chronic stress conditions.

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