Chapter 7

GENERAL DISCUSSION
Chapter 7 General discussion

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1. Summary of results

The objective of this thesis was to find causal factors underlying individual differences in susceptibility to stress and stress-related psychopathologies. These individual differences were studied in two lines of mice, the LAL and SAL mice, that show distinctly different behavioural strategies towards environmental stimuli. It was hypothesized that this difference in behavioural coping style is associated with differences in stress reactivity and hence a differential susceptibility to stressors. This thesis focussed on the regulation of the HPA and 5-HT systems and on differences in hippocampal plasticity under baseline conditions as well as in response to stress. Further knowledge about how individuals can differ in their responses to stressors can be helpful to understand the relationship between stress and stress-related mood disorders like depressive illness.

To investigate whether a difference at the behavioural level was associated with a difference in HPA axis (re)activity, the LAL and SAL mice were characterized for their neuroendocrine pattern under baseline and acute stress conditions (chapter 2). LAL mice showed no fluctuation of plasma corticosterone levels around the circadian peak and had lower basal ACTH levels than SAL mice. This latter finding suggests a line difference in adrenocortical sensitivity to ACTH. At a central level, no basal line differences were found in the mRNA expression of hippocampal GR and MR or hypothalamic CRH. Acute stress (forced swimming for 5 min) induced higher immobility behaviour in LAL mice, which is consistent with the ‘passive’ behavioural coping style displayed by these mice. This higher immobility behaviour in LAL mice was further associated with an enhanced and prolonged corticosterone response compared to SAL mice, while absolute ACTH levels did not differ between the mouse lines. Moreover, only in LAL mice, hippocampal MR mRNA (but not GR) and hypothalamic CRH mRNA were significantly higher at 24 h post-stress. This clearly indicates that a genetic trait in behaviour is associated with an idiosyncratic pattern of HPA activity, and greater responsiveness of physiological and molecular stress markers in LAL mice.

As LAL and SAL mice displayed a differential regulation of the HPA axis after exposure to an acute stressor, the experiment described in chapter 3 was designed to examine line differences in response to two chronic stressors (defeat stress and sensory contact stress). The defeat stressor (21 daily defeats) led to increases in stress-markers in both lines, although the pattern of changes was specific for each line. Interestingly, continuously living in sensory contact with an aggressive SAL male for 25 days induced long-lasting stress symptoms in LAL mice but not in SAL mice. These stress symptoms included long-lasting body weight loss, increased plasma ACTH and corticosterone levels, thymus involution and lower hippocampal MR mRNA expression. Despite these clear stress-induced changes in HPA system, behavioural parameters were relatively unresponsive to
sensory contact stress as well as defeat stress. Nevertheless, the differential response, in particular, to sensory contact stress indicates that LAL mice displayed higher stressor susceptibility.

Chapter 4 shows that 5 days of sensory contact stress already induced a differential response in LAL and SAL mice. In agreement with the 25 day model, LAL mice showed a higher stressor susceptibility, as indicated by higher corticosterone levels, adrenal hypertrophy and several central changes. Interestingly, the pattern of central changes in LAL mice after 5 days (increased hypothalamic CRH and hippocampal dentate gyrus GR mRNA expression, and decreased hippocampal 5-HT\textsubscript{1A} receptor mRNA expression) was distinctly different than that found after 25 days (decreased MR mRNA expression). This suggests that the pattern of stress symptoms is specific for the duration of the stressor. In SAL mice, markers of the HPA system suggest that sensory contact stress had only transient effects. It was also shown that the magnitude of these stress effects was determined by the type of opponent (SAL male vs. LAL male vs. isolation). Isolation caused already several peripheral changes. Yet, exposure to a SAL male was the most severe stressor for LAL mice, whereas exposure to a LAL male produced most changes in SAL mice. This indicates that a differential stressor susceptibility in LAL and SAL mice depends on the type of opponent in the sensory contact model.

Both stress and elevated circulation of glucocorticoids have been identified as negative regulators of adult hippocampal neurogenesis. We therefore tested in chapter 5 the hypothesis that a difference in HPA regulation LAL and SAL mice is associated with a difference in hippocampal cell proliferation. To visualize cell proliferation, two methods were used: injections with BrdU which is incorporated in dividing cells and the endogenous proliferation marker Ki-67. Using three daily injections with BrdU, we found that the number of proliferating cells were almost twice as low in LAL than in SAL mice. However, these injections resulted in significantly higher corticosterone levels in LAL compared to SAL mice, which likely affected the cell proliferation rate in the LAL mice. A subsequent experiment with the endogenous marker Ki-67 showed that under baseline conditions, LAL mice had slightly but significantly lower cell proliferation (85%) than SAL mice. By subjecting the mice to forced swim stress, cell proliferation was suppressed in LAL mice but not in SAL mice. Although forced swim stress induced higher corticosterone levels in LAL mice compared to control LAL mice, no significant difference was present between the LAL and SAL mice. This suggests the involvement of glucocorticoids in the stress-induced suppression of cell proliferation in LAL mice, while cell proliferation in SAL mice was resistant to stress-induced down-regulation.
In chapter 6 it was investigated whether the difference between LAL and SAL mice in forebrain 5-HT_{1A} receptor expression was associated with a difference in 5-HT metabolism. It was also investigated whether the line difference in forced swimming behaviour could be changed by administration of 5-HT_{1A} receptor agonists. First, it was confirmed that LAL mice had lower 5HT_{1A} receptor expression and binding capacity in the hippocampus than SAL mice, whereas no difference was found in lateral septum, prefrontal cortex or dorsal raphe nucleus. Furthermore, 5-HT content was slightly higher in LAL than in SAL mice, in particular during the light phase, reaching significance in the brain stem. In addition, LAL mice showed lower 5-HT turnover in several brain regions, reaching significance in striatum and amygdaloid region. This suggests lower 5-HT activity in specific brain regions in LAL compared to SAL mice. In the forced swim test, LAL mice showed more immobility and less climbing behaviour than SAL mice. This behavioural line difference was abolished by treatment with the full 5-HT_{1A} receptor agonist 8-OH-DPAT (2 and 5 mg/kg). The presynaptic 5-HT_{1A} autoreceptor agonist S-15535 (20 mg/kg) induced a similar change in forced swimming behaviour in SAL mice but did not affect the behaviour in LAL mice. This suggests that the behavioural change was mediated via activation of postsynaptic 5-HT_{1A} receptors in LAL mice and via presynaptic 5-HT_{1A} receptors in SAL mice. This was further confirmed by the observation that 8-OH-DPAT decreased 5-HT turnover in seven out of nine brain regions in SAL but not in LAL mice. 5-HT turnover after forced swimming was lower in LAL than in SAL mice in septum, parietal and occipital cortex and brain stem. Thus, the differential effects of 8-OH-DPAT and S-15535 on forced swimming behaviour and the line differences in 5-HT metabolism suggests a differential functioning or activation of the 5-HT system between LAL and SAL mice.

Our results demonstrate that a line difference in behavioural coping style is associated with a difference in HPA (re)activity, hippocampal plasticity, 5-HT metabolism and 5-HT_{1A} receptor functioning. In general, the impact of stress on several markers of the HPA system, behaviour, hippocampal cell proliferation and 5-HT system showed a higher stressor susceptibility in LAL mice. In the following paragraph, these results will be discussed in more detail. In the last paragraph, possible implications of these line differences for stress adaptation will be discussed as well as whether these mice can serve as an animal model to study depression-like symptoms.
2. Line differences under basal and acute stress conditions

2.1. HPA axis (for overview see Table 1)

Differences in HPA regulation were found between low-aggressive LAL and high-aggressive SAL mice on the level of plasma ACTH and corticosterone under baseline conditions as well as after exposure to an acute stressor. Remarkable was the finding of consistently lower ACTH levels in naïve LAL mice, while corticosterone levels were not different from SAL mice (chapter 2). This suggests a difference in adrenocortical sensitivity to ACTH. This difference was still present after exposure to an acute stressor, as LAL and SAL mice showed a similar stress-induced increase in ACTH (15 min after forced swimming for 5 min), but stress-induced corticosterone levels were significantly higher in LAL than in SAL mice (chapter 2). A difference in adrenocortical sensitivity to ACTH was also found in selection lines of rats. Rats genetically selected for low and high apomorphine susceptibility (Rots et al., 1995, 1996) as well as Roman low- and high-avoidance rats (Walker et al., 1989) showed a similar difference in adrenocortical sensitivity to ACTH as the LAL and SAL mice. Interestingly, LAL and SAL mice also differ in apomorphine susceptibility and in avoidance behaviour (Benus et al., 1989, 1991a). This suggests a possible link between certain behavioural, neurochemical and neuroendocrine patterns, although interpretation of these apparent correlates should be taken by some caution.

The mechanism underlying this line difference in adrenocortical sensitivity to ACTH is not clear. It is known, however, that several factors can influence the responsiveness of the adrenal gland to ACTH. Among these are several peptides such as β-endorphin, dynorphin 1-17 and vasoactive intestinal peptide (Guaza et al., 1986; Bodnar et al., 1997), but also splanchnic innervation of the adrenal (Edwards and Jones, 1993) and release of CRH at the level of the adrenal gland (Edwards and Jones, 1988; Van Oers et al., 1992) can control the sensitivity of the adrenal to ACTH. It would, therefore, be of interest to study the role of, for example, endogenous CRH in controlling adrenal responsiveness to ACTH in LAL and SAL mice. A better understanding of the mechanisms underlying the complex interaction between ACTH and corticosterone may also be helpful for situations were a decrease in corticosteroid levels is desired.

In addition to the relatively reduced ACTH release and signs of adrenocortical hyperresponsiveness, LAL mice showed some differences in baseline and stress-induced release of corticosterone compared to SAL mice. First, it was found that LAL mice showed less day-night variation in corticosterone release around the circadian peak than SAL mice (chapter 2). These circadian differences might be due to altered mineralocorticoid and glucocorticoid receptor function.
Second, LAL mice showed higher circulating corticosterone concentrations in response to an acute stressor (forced swimming for 5 min) (chapter 2). This is in agreement with another study in which LAL and SAL mice were exposed to novelty stress (Van Riel et al., 2002). Also in several other species (rats, chicken and pigs) it was shown that animals with a ‘passive’ coping style, showed a higher activation of the HPA axis than ‘active’ coping animals (De Boer et al., 1990; Korte et al., 1997; Schouten and Wiegant, 1997). The degree of activation of the HPA axis was found to be related to the intensity of stress experienced by animals (Hennessy and Levine, 1978; Koolhaas et al., 1997). This suggests that ‘passive’ coping animals like the LAL mice perceive a higher intensity of the same stressor than ‘active’ coping animals like the SAL mice. This bias for interpreting stressful stimuli as potentially threatening has also been observed in several other selection lines of rats (Overstreet et al., 1992; Steimer et al., 1997; Landgraf and Wigger, 2002), suggesting that the correlation between coping style (behavioural stress response) and neuroendocrine stress responses holds across species.

Third, LAL mice showed a prolonged stress-induced increase in circulating corticosterone concentrations compared to SAL mice (chapter 2, 5). This may indicate an impaired GR-mediated termination of the stress response. GR dysfunctioning could also clarify the higher weights of thymus and spleen (GR-containing organs) in LAL mice than in SAL mice (chapter 2, 4). Although no line difference in GR mRNA expression was found in hippocampus nor in hypothalamus (chapter 2, 4), this does not exclude a possible difference at GR protein level or GR function. Further research is required to corroborate possible GR dysfunctioning in the LAL mice.

**Table 1.** Overview of the differences in HPA regulation between LAL and SAL mice under baseline conditions and after exposure to an acute stressor (forced swimming for 5 min). Plasma ACTH and corticosterone (CORT) were measured under baseline conditions and shortly (15 and 90 min) after exposure to an acute stressor. The mRNA expression was measured for hippocampal MR and GR as well as the ratio MR:GR and CRH in the hypothalamic paraventricular nucleus (PVN), under baseline conditions and 24 h after an acute stressor. Arrows indicate a significant difference (lower , or higher ) compared to SAL mice or compared to control LAL or SAL (con) mice. No arrow indicates that there is no significant difference.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plasma</th>
<th>Hippocampus</th>
<th>PVN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ACTH</td>
<td>CORT</td>
<td>MR</td>
</tr>
<tr>
<td>LAL vs. SAL</td>
<td>Baseline</td>
<td>↓ less fluctuation</td>
<td>↑</td>
</tr>
<tr>
<td>LAL vs. con</td>
<td>Acute stress</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>SAL vs. con</td>
<td>Acute stress</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>LAL vs. SAL</td>
<td>Acute stress</td>
<td>↑</td>
<td></td>
</tr>
</tbody>
</table>
* see also Fig. 1.
Under baseline conditions, no line differences were found in transcript levels of hippocampal MR, GR or hypothalamic CRH (chapter 2), nor in GR in the hypothalamus (chapter 4) or central amygdala (Feldker and Veenema, unpublished observation). However, in response to an acute stressor (forced swimming for 5 min), LAL but not SAL mice showed an up-regulation in hippocampal MR mRNA and hypothalamic CRH mRNA when measured 24 h later (chapter 2). Due to the increase in MR mRNA, hippocampal MR:GR ratio was also significantly higher in LAL mice (see Fig. 1). This stress-induced increase in hippocampal MR is in agreement with another study, in which increased hippocampal MR immunoreactivity was observed 24 h following exposure of rats to forced swimming (Gesing et al., 2001). Hippocampal MRs display a tonic inhibitory function on the activity of the HPA axis (De Kloet and Reul, 1987; De Kloet et al., 1998) and have been implicated in the control of behavioural reactivity (Oitzl et al., 1994). In this framework, an increase in hippocampal MR mRNA expression is thought to have implications for changes in the regulatory control of the HPA axis and behavioural coping in LAL mice. As a consequence, LAL and SAL mice may develop a distinctly different neuroendocrine and behavioural phenotype after chronic exposure to uncontrollable stress.

Fig. 1. Effect of forced swimming stress on MR:GR ratio in hippocampus (dentate gyrus and CA1 subregion) of LAL and SAL mice measured 24 h post-stress. The MR/GR ratio (determined by dividing the optical density of the mRNA expression of MR by the mRNA expression of GR per mouse) showed a significant treatment effect ($F_{1,21} = 8.010$, $P = 0.010$, pairwise comparisons following univariate ANOVA). Forced swim stress induced a significant increase in MR:GR ratio in LAL mice compared to SAL mice ($P < 0.05$) and LAL controls ($P < 0.01$).
In general, it can be concluded that LAL and SAL male mice differ in setpoint and responsiveness of the HPA axis, as shown by differences in ACTH release, adrenocortical responsiveness, circadian and stress-induced corticosterone release, and MR and CRH expression profiles. In particular the fast stress-induced changes in molecular markers of the HPA axis in LAL compared to SAL mice may indicate a line difference in the capacity to cope with stress.

2.2. 5-HT system

2.2.1. 5-HT$_{1A}$ receptor gene expression and function

It was reported earlier that LAL mice had lower forebrain 5-HT$_{1A}$ receptor gene expression and binding than SAL mice, whereas no line difference was observed in the dorsal raphe nucleus (Korte et al., 1996). We confirmed that LAL mice had lower 5-HT$_{1A}$ receptor gene expression and binding capacity in the hippocampus than SAL mice, whereas no difference was found in dorsal raphe nucleus (chapter 3, 4, 6) (Table 2). Also, two other studies showed a similar line difference in the hippocampus (Van Riel et al., 2002; Feldker et al., 2003a). We could, however, not establish a line difference in frontal cortex or lateral septum (chapter 6) as was reported by Korte and colleagues (1996). This was likely due to a difference in time of the day prior to tissue preparation, which was in the light phase in our experiment and in the dark phase in the experiment of Korte et al. (1996). The lower 5-HT$_{1A}$ receptor gene expression in LAL mice was associated with an attenuated functional response to 5-HT in CA1 hippocampal neurons (Van Riel et al., 2002). Moreover, an attenuated responsiveness of postsynaptic 5-HT$_{1A}$ receptors in LAL mice was also found by measuring the hypothermic response mediated via postsynaptic 5-HT$_{1A}$ receptor activation (Van der Vegt et al., 2001). Interestingly, a similar reduced sensitivity of postsynaptic 5-HT$_{1A}$ receptors was found in rats with low trait aggression (derived from an unselected strain of wild-type rats) compared to rats with high trait aggression (Van der Vegt et al., 2001). These data demonstrate that low levels of aggression are linked to low postsynaptic 5-HT$_{1A}$ receptor sensitivity.

A difference in 5-HT$_{1A}$ receptor gene expression and function might be due to a difference in the release of HPA hormones. In the study of Korte et al. (1996) and Van Riel et al. (2002), the lower 5-HT$_{1A}$ receptor gene expression in LAL mice was associated with higher corticosterone levels than in SAL mice. Corticosteroids were found to inhibit hippocampal 5-HT$_{1A}$ receptor mRNA expression (Chalmers et al., 1993; Meijer et al., 1994). Adrenalectomy induces an up-regulation of hippocampal 5-HT$_{1A}$ receptor mRNA expression (De Kloet et al., 1986; Chalmers
et al., 1993), whereas chronically elevated corticosterone levels decreases hippocampal 5-HT\textsubscript{1A} receptor binding and gene expression (Watanabe et al., 1993; Meijer et al., 1997; Lopez et al., 1998; Veenema, unpublished) This suggests the involvement of corticosterone in the line difference in 5-HT\textsubscript{1A} receptor gene expression. In contrast, our studies (Chapter 3, 4 and 6) and the study by Feldker et al. (2003a) observed lower hippocampal 5-HT\textsubscript{1A} receptor gene expression in LAL mice without a line difference in corticosterone levels. Considering, however, the bias in HPA responsiveness between the LAL and SAL mice, the possibility of corticosterone-induced changes in 5-HT\textsubscript{1A} receptor gene expression can not be excluded.

To further investigate a line difference in 5-HT\textsubscript{1A} receptor functioning, the effects of two 5-HT\textsubscript{1A} receptor agonists were studied on the behavioural response of LAL and SAL mice in the forced swim test (see Table 3). In this test, LAL mice showed high immobility behaviour whereas SAL mice displayed high active behaviour (Chapter 2, 3, 6). The forced swim test was developed by Porsolt and colleagues (1977a,b), and is nowadays a widely used animal model in depression research as a screen for antidepressant treatments (Sanchez and Meier, 1997; Lucki, 1997; Page et al., 1999; Porsolt, 2000). In addition to antidepressants, also 5-HT\textsubscript{1A} receptor agonists were found to decrease immobility behaviour (Wieland and Lucki, 1990; Singh and Lucki, 1993; Schreiber and De Vry, 1993; Detke et al., 1995; O’Neill and Conway, 2001). We showed that acute treatment with the full 5-HT\textsubscript{1A} receptor agonist, 8-OH-DPAT, abolished the behavioural line differences by reducing immobility behaviour in LAL mice and reducing swimming behaviour in SAL mice (Chapter 6).

As 8-OH-DPAT can bind to both post- and presynaptic 5-HT\textsubscript{1A} receptors, the preferential presynaptic 5-HT\textsubscript{1A} receptor agonist S-15535 was used to discriminate between post- and presynaptic effects. If the behavioural effect of 8-OH-DPAT was induced by predominant activation of presynaptic receptors, a similar behavioural change in LAL and SAL mice was expected by acute treatment with S-15535. Forced swimming behaviour in LAL mice was, however, not affected by S-15535, whereas the same ligand reduced climbing behaviour in SAL mice (Chapter 6). This suggests that the decrease in immobility behaviour by 8-OH-DPAT in LAL mice was induced by predominant activation of postsynaptic 5-HT\textsubscript{1A} receptors. In contrast to LAL, predominant activation of presynaptic 5-HT\textsubscript{1A} autoreceptors by 8-OH-DPAT and S-15535 likely mediated the decrease of active behaviour in the SAL mice. Interestingly, also others have reported differences in the behavioural effects of antidepressant drugs, which seem to depend on line or strain differences in the behavioural response to forced swimming (Godfrey et al., 1997; Lahmame and Armario, 1996; Lopez-Rubalcava and Lucki, 2000; Lucki et al., 2001; Tejani-Butt et al., 2003). Thus, our data may indicate that a differential behavioural response to forced swimming is associated with a differential homeostatic control of the 5-HT neuron.
Table 2. Overview of the differences between LAL and SAL mice in $5\text{-HT}_{1\text{A}}$ receptor gene expression and binding in hippocampus (HI), frontal cortex (FC), lateral septum (LS) and dorsal raphe nucleus (DRN), and in 5-HT metabolism measured in several brain regions under baseline conditions. Arrows indicate a significant difference (lower ↓, or higher ↑) compared to SAL mice. No arrow means no significant difference.

<table>
<thead>
<tr>
<th>5-HT$_{1\text{A}}$ receptor</th>
<th>5-HT metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>HI FC LS DRN</td>
<td>5-HIAA 5-HT 5-HIAA/5-HT</td>
</tr>
<tr>
<td>LAL vs. SAL ↓</td>
<td>↑*</td>
</tr>
<tr>
<td>* only in brain stem, * only in striatum and amygdaloid region</td>
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</table>

Table 3. Overview of the differences between LAL and SAL mice in forced swimming behaviour, in the behavioural effects of the full 5-HT$_{1\text{A}}$ receptor agonists, 8-OH-DPAT and of the preferential presynaptic 5-HT$_{1\text{A}}$ receptor agonist, S-15535, and the effects of 8-OH-DPAT on forced swim induced 5-HT metabolism in several brain regions. Arrows indicate a significant difference (lower ↓, or higher ↑) compared to SAL mice or compared to control LAL or SAL (con) mice. The effect of S-15535 on brain 5-HT metabolism was not measured. No arrows indicate that there is no significant difference.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Behaviour</th>
<th>5-HT metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>LAL vs. SAL ↑ immobility</td>
<td>5-HIAA ↑* 5-HT ↓*</td>
</tr>
<tr>
<td>8-OH-DPAT</td>
<td>LAL vs. SAL no line difference</td>
<td>5-HIAA ↑* 5-HT ↓*</td>
</tr>
<tr>
<td></td>
<td>LAL vs. con ↓ immobility</td>
<td>5-HIAA ↑* 5-HT ↓*</td>
</tr>
<tr>
<td></td>
<td>SAL vs. con ↓ climbing</td>
<td>5-HIAA ↑* 5-HT ↓*</td>
</tr>
<tr>
<td>S-15535</td>
<td>LAL vs. SAL ↑ immobility</td>
<td>5-HIAA ↑* 5-HT ↓*</td>
</tr>
<tr>
<td></td>
<td>LAL vs. con no effect</td>
<td>5-HIAA ↑* 5-HT ↓*</td>
</tr>
<tr>
<td></td>
<td>SAL vs. con ↓ climbing, ↑ immobility</td>
<td>5-HIAA ↑* 5-HT ↓*</td>
</tr>
<tr>
<td>* in several brain regions, * only in brain stem</td>
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2.2.1. 5-HT metabolism

In this thesis it was suggested that the lower gene expression of 5-HT$_{1\text{A}}$ receptors in LAL mice could have been an adaptive compensatory response to higher levels of synaptic 5-HT. A previous study already showed that SAL mice had lower whole brain 5-HT levels compared to SAL mice (Olivier et al., 1990).
This also agrees with the general view that high aggressiveness is linked to low 5-HT metabolism. We examined further in which brain regions differences in 5-HT levels between LAL and SAL mice were present. In agreement with Olivier et al. (1990), we found that 5-HT content in most brain regions was slightly higher in LAL than in SAL mice, but this difference was only significant in brain stem (chapter 6) (Table 2). 5-HT turnover was found to be significantly lower in striatum and amygdaloid region in LAL compared to SAL mice (Table 2). Thus, in most brain regions, levels of 5-HT, 5-HIAA and 5-HT turnover were not significantly different between LAL and SAL mice. It is, therefore, unlikely that the observed difference in 5-HT\textsubscript{1A} properties between SAL and LAL mice is an adaptive compensatory reaction to changes in 5-HT metabolism. As a consequence, the suggested link between low 5-HT metabolism and high aggressiveness could not be confirmed in these high-aggressive SAL mice. Obviously, more delicate methods, such as microdialysis, need to be applied to further test this. Nevertheless, even in these high-aggressive mice, no clear signs of reduced 5-HT metabolism could be found, suggesting a rather refined involvement of 5-HT in aggression.

The distinct behavioural profiles of LAL and SAL mice in the forced swim test and the differential behavioural effect of the two 5-HT\textsubscript{1A} receptor agonists, 8-OH-DPAT and S-15535, in LAL and SAL mice (chapter 6), could be due to endogenous differences in stress-induced brain 5-HT metabolism and 5-HT\textsubscript{1A} receptor sensitivity. Indeed, a line difference was found for 5-HT metabolism in response to forced swimming. LAL mice showed higher 5-HT content and attenuated 5-HT turnover in several brain regions compared to SAL mice (chapter 6) (Table 3). This suggests a diminished activation of the 5-HT system in LAL mice in response to forced swimming.

Furthermore, acute treatment with the 5-HT\textsubscript{1A} receptor agonist, 8-OH-DPAT, decreased the 5-HT turnover in most brain regions in SAL mice subjected to forced swimming, whereas it had no effect on 5-HT metabolism in LAL mice (chapter 6) (Table 3). This further supports the idea that the anti-climbing effect of 8-OH-DPAT in SAL mice was induced by predominant activation of presynaptic 5-HT\textsubscript{1A} receptors, resulting in a decrease in 5-HT metabolism. In contrast, the absence of a change in 5-HT metabolism in LAL mice suggests that the anti-immobility effect of 8-OH-DPAT was mediated by predominant postsynaptic 5-HT\textsubscript{1A} receptor activation.

In conclusion, clear line differences in the expression and function of postsynaptic 5-HT\textsubscript{1A} receptors and in 5-HT metabolism were found, indicating that the passive coping strategy of LAL mice is associated with lower brain 5-HT and 5-HT\textsubscript{1A} receptor functioning in particular in response to acute forced swim stress compared to the active coping strategy of SAL mice. It will be of interest for future research to study the effect of chronic stress on these markers of the 5-HT system.
2.3. Hippocampal plasticity

The hippocampus plays a critical role in declarative, spatial, and contextual memory as well as in emotional processing. This structure is also a major target for glucocorticoids (Sloviter et al., 1989; Joëls and De Kloet, 1990; Joëls et al., 1991; Pavlides et al., 1994; De Kloet et al., 1998). Furthermore, structural changes in the hippocampus were found to be induced by chronic stress and excess levels of glucocorticoids (McEwen, 2000a,b, 2001; Sapolsky 2000, 2001). It is likely that other neurotransmitter systems like the 5-HT system participate along with circulating glucocorticoids in inducing these structural changes. Hippocampal plasticity is, therefore, an interesting read-out system to study the consequences of the profound differences in HPA functioning (chapter 2, 3, 4), 5-HT system (Korte et al., 1996; Van Riel et al., 2001; chapter 6), and behavioural traits in LAL and SAL mice. First, a comparison was made between the expression levels of hippocampal transcriptomes of LAL and SAL mice. Second, the production of new hippocampal cells was studied under basal and acute stress conditions in these mice.

2.3.1. Hippocampal gene expression profile (Feldker et al., 2003a,b)

To investigate differences in hippocampal gene expression which could be related to the line differences found in behavioural traits, HPA axis and 5-HT system, a hippocampal gene expression profile was generated in LAL and SAL mice by using a large scale gene expression profiling method Serial Analysis of Gene Expression (SAGE) and high-density oligonucleotide arrays (GeneChips; Affymetrix). SAGE generated almost 30 000 genes of which 191 genes were differentially expressed between LAL and SAL mice (Feldker et al., 2003a) (see also Table 4 and Fig. 2). The GeneChips contained approximately 12 000 genes and ESTs (expressed sequence tags) of which 165 were differentially expressed between LAL and SAL mice (Feldker et al., 2003b) (see also Table 4 and Fig. 2).

It is likely, however, that the total number of genes that were found to be differentially expressed between LAL and SAL mice, is an underestimation of the real number of differentially expressed genes. Both techniques generated expression profiles biased to abundant and medium abundant genes and failed to detect genes expressed at low levels in a complex brain structure such as the hippocampus (for reviews see Evans et al., 2002, Feldker et al., 2003c). In the GeneChips studies, almost 75% of the probe sets were considered non-reliable mainly due to too low signal intensities, among which are, for example, 5-HT receptors (Feldker et al., 2003b). Many neuron-specific genes were, therefore, not represented in the generated expression profile (Feldker et al., 2003a,b). Thus, regardless of their powerful abilities for monitoring thousands of transcript levels simultaneously, detection of low-abundance transcripts, of which many have great
relevance to biological function in brain, is not yet possible using these advanced techniques.

Table 4. Overview of the two approaches, i.e., the large scale gene expression profiling techniques, SAGE and GeneChip analysis, to identify differentially expressed hippocampal genes between LAL and SAL mice.

<table>
<thead>
<tr>
<th></th>
<th>SAGE</th>
<th>GeneChips</th>
</tr>
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<tbody>
<tr>
<td>Number of mice</td>
<td>LAL n = 14 pooled</td>
<td>LAL n = 5</td>
</tr>
<tr>
<td></td>
<td>SAL n = 14 pooled</td>
<td>SAL n = 5</td>
</tr>
<tr>
<td>Number of genes</td>
<td>29 727</td>
<td>12 000</td>
</tr>
<tr>
<td>Reliable detected</td>
<td>598 (&gt; 10 times expressed)</td>
<td>2 865 (dep. on signal intensity)</td>
</tr>
<tr>
<td>Differentially expressed genes</td>
<td>191 ($P &lt; 0.05$)</td>
<td>165 ($P &lt; 0.01, &gt; 1.3$ fold change)</td>
</tr>
<tr>
<td></td>
<td>126 ↑ in LAL</td>
<td>148 ↑ in LAL</td>
</tr>
</tbody>
</table>

Fig. 2. Overview of the differentially expressed hippocampal genes between LAL and SAL mice using SAGE and GeneChip analysis.
Despite this limitation, both SAGE and GeneChips revealed some interesting genes of which the majority were expressed at higher levels in LAL mice. Among these were numerous cytoskeleton genes, such as coflin and several tubulin isotypes which were higher expressed in LAL than in SAL mice (Feldker et al., 2003a,b). This differential expression of several cytoskeleton genes may imply a difference in hippocampal morphology between LAL and SAL mice. Consistent with this, was the reported larger hippocampal distribution of mossy fibres terminal fields in LAL than in SAL mice (Sluyter et al., 1994).

Furthermore, a higher expression of several calmodulin-related genes (such as calmodulin and neurogranin) and genes encoding components of a mitogen activated protein kinase (MAPK) cascade (raf-related oncogene and MAPK1/ERK2) was found in LAL compared to SAL mice (Feldker et al., 2003a). As proteins, these genes play an important role in specific signal transduction pathways. MAPK-related genes have been found to be critical for neuronal plasticity and memory consolidation (Impey et al., 1999) and calmodulin-related genes play an important role as second messengers in, for example, 5-HT1A receptor mediated activation of extracellular regulated kinases (Della Rocca et al., 1999). A number of these differentially expressed genes (alpha-tubulin, coflin, raf-related oncogene and ERK2) were validated by in situ hybridisation (Feldker et al., 2003a).

Although most differentially expressed genes were higher (up to three-fold) in LAL than in SAL mice, GeneChips revealed one EST corresponding to growth arrest specific 5 (gas5) which was 8 times lower expressed in LAL than in SAL mice (Feldker et al., 2003b). The gas5 gene is expressed at high levels in the growth arrest (or G0) phase of the cell cycle (Schneider et al., 1988). Although the regulation of the expression of this gene was studied in respect to cell growth and differentiation (Ciccarelli et al., 1990), its precise function is still unclear. If, however, gas5 is involved in the regulation of cell growth and morphology, the differential expression of the gas5 gene between LAL and SAL mice may have implications for differences in these processes. Using in situ hybridisation the differential expression of gas5 was observed throughout the entire brain (Feldker et al., 2003b), which opens the possibility that some morphological differences between LAL and SAL mice are not restricted to the hippocampus.

At present it is unclear whether the observed LAL:SAL differences in gene expression found by SAGE and GeneChips, persist at protein level and whether they are associated with the line differences found in hippocampal morphology and behavioural and neuroendocrine phenotype. Nevertheless, the higher expression of several cytoskeleton and signal transduction genes in LAL mice compared to SAL mice strongly suggests a difference in hippocampal plasticity, which can be relevant under conditions of stress.
2.3.2. Hippocampal cell proliferation

In most brain regions, the production of neurons only occurs during a discrete developmental period. However, in the hippocampal dentate gyrus the production of granule neurons continues throughout adult life. This has been shown in several mammalian species from rodents to humans (Altman and Das, 1967; Kaplan and Hinds, 1977; Rakic and Nowakowski 1981; Gueneau et al., 1982; Gould et al., 1997, 1998; Eriksson et al., 1998). Although the precise function of these new neurons remains unclear, the regulation of cell proliferation and survival by experience, environmental enrichment, running, stress, learning and memory, hormones and aging (Gould et al., 1992, 1997, 1999a; Kemperman et al., 1997, 1998; Van Praag et al., 1999; Tanapat et al., 1999, 2001; Shors et al., 2001, 2002; Heine et al, 2003), has lead scientists to suggest that these new granular neurons might contribute to hippocampal function and plasticity.

Under baseline conditions, LAL mice had slightly lower (85%) hippocampal cell proliferation rate than SAL mice (chapter 5). It was further found that three daily i.p. injections augmented the line difference in hippocampal cell proliferation rate (chapter 5). This difference was accompanied by higher plasma corticosterone concentrations in LAL mice 24 h after the last injection (chapter 5). This confirms the earlier found higher HPA reactivity in the LAL mice (chapter 2) and supports the idea that LAL mice perceive a higher intensity of the same stressor than SAL mice. As glucocorticoids can suppress the proliferation rate of newborn cells (Gould et al., 1997, 1998; Tanapat et al., 2001), the higher corticosterone concentrations may have induced a further suppression of hippocampal cell proliferation in LAL mice.

Using another acute stressor (forced swim stress for 5 min) it was demonstrated that the number of proliferating cells were suppressed in LAL but not in SAL mice (chapter 5). In LAL mice, this suppression may have partly been induced by the higher and prolonged release of plasma corticosterone compared to control LAL mice (chapter 5). However, corticosterone concentrations between stressed LAL and SAL mice were not different. This suggests a resistance in SAL mice to the negative effects of corticosterone on cell proliferation. It was proposed that this could be mediated by simultaneous activation of positive regulators of adult neurogenesis. The 5-HT\textsubscript{1A} receptor is, in this respect, an interesting candidate. Activation of 5-HT\textsubscript{1A} receptors increases adult hippocampal cell proliferation (Brezun and Daszuta, 1999; Gould, 1999b; Jacobs et al., 1998, 2000; Jacobs, 2002; Radley and Jacobs, 2002), and higher hippocampal 5-HT\textsubscript{1A} receptor gene expression and function was found in SAL versus LAL mice (Korte et al., 1996; chapter 3, 4, 6; Van Riel et al., 2002). Further research is required to confirm this potential role of the hippocampal 5-HT\textsubscript{1A} receptor in preventing the stress-induced decrease in cell proliferation in SAL mice.
In conclusion, LAL mice showed a higher expression of several hippocampal genes involved in hippocampal morphology and plasticity compared to SAL mice. In addition, LAL mice showed a slightly lower hippocampal cell proliferation rate than SAL mice, which was further amplified after exposure to acute stressors. Although this latter finding may seem to be inconsistent with the hippocampal gene expression profile, it rather shows the ability of LAL mice to respond to a stressor. Thus, the line differences in hippocampal transcriptomes and cell proliferation may both indicate a higher plasticity of the hippocampus in LAL mice. It would, therefore, be of interest to study the consequences of these differences for cognitive performance as well as for coping with chronic stressors.

3. Line differences after exposure to chronic stressors

As suggested earlier, LAL and SAL mice may develop a distinctly different neuroendocrine and behavioural phenotype after chronic exposure to uncontrollable stress. The higher responsiveness of molecular markers of the HPA system, the higher expression of several hippocampal genes together with the higher behavioural flexibility, would suggest that LAL mice may also show a higher amplitude of changes in response to chronic stressors than SAL mice. Alternatively, the higher corticosterone response, signs of 5-HT hypofunction and lower hippocampal cell proliferation rate in LAL mice may result in a differential susceptibility to chronic stressors in LAL compared to SAL mice. The ability to cope with different chronic psychosocial stressors was, therefore, studied in these two mouse lines.

3.1. Line differences in stressor susceptibility

3.1.1. Social isolation

Social isolation of rodents (by housing them single in a cage) has been shown by others to affect a number of physiological variables and elevates plasma corticosterone levels (Brain and Berton, 1979; Parker and Morinan, 1986). However, ambiguities in the literature on isolation stress have also been reported (Benton and Brain, 1981; Misslin et al., 1982; Holson et al., 1991), which are likely attributed to variables in strain and test conditions (Benton and Brain, 1981; Misslin et al., 1982). This variability in the results emphasizes that isolation from a conspecific is a complex phenomenon, involving an alteration of social environment, increased metabolic demands of temperature maintenance (which is particularly relevant for mice) and decreased sensory stimulation.
Social isolation for a period of 5 days, induced a decrease in body weight in LAL as well as in SAL mice, while thymus involution and elevated corticosterone levels were only seen in LAL mice (Chapter 4). Interestingly, after one day of social isolation, corticosterone levels were higher in SAL than in LAL mice. This may indicate transient activation of the HPA axis in SAL mice and a delayed stress response in LAL mice. Despite the changes at the physiological level, social isolation had no effect on central molecular HPA markers in both mouse lines (see Table 5).

**Table 5.** Effect of 5 days of social isolation on the mRNA expression (in arbitrary units) of MR and GR in the CA1 region and the dentate gyrus of the hippocampus, and of GR and CRH in the paraventricular nucleus (PVN) of the hypothalamus in LAL and SAL male mice. No line or treatment effect was found (ANOVA).

<table>
<thead>
<tr>
<th>Region</th>
<th>LAL control</th>
<th>LAL isolation</th>
<th>SAL control</th>
<th>SAL isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR CA1 region</td>
<td>77.5 ± 5.7</td>
<td>64.3 ± 7.1</td>
<td>77.6 ± 4.6</td>
<td>81.1 ± 4.6</td>
</tr>
<tr>
<td>Dentate gyrus</td>
<td>82.6 ± 5.4</td>
<td>75.4 ± 7.3</td>
<td>84.9 ± 4.4</td>
<td>89.0 ± 5.0</td>
</tr>
<tr>
<td>GR CA1 region</td>
<td>49.3 ± 1.9</td>
<td>47.9 ± 1.0</td>
<td>48.1 ± 2.4</td>
<td>51.7 ± 1.6</td>
</tr>
<tr>
<td>Dentate gyrus</td>
<td>39.2 ± 1.2</td>
<td>43.4 ± 1.3</td>
<td>41.9 ± 2.5</td>
<td>42.9 ± 1.7</td>
</tr>
<tr>
<td>PVN</td>
<td>61.6 ± 1.9</td>
<td>55.1 ± 2.9</td>
<td>54.6 ± 3.2</td>
<td>51.2 ± 5.3</td>
</tr>
<tr>
<td>CRH PVN</td>
<td>67.5 ± 7.3</td>
<td>76.0 ± 4.8</td>
<td>66.4 ± 4.2</td>
<td>56.8 ± 9.6</td>
</tr>
</tbody>
</table>

5-HT$_{1A}$ receptor mRNA expression was, however, decreased in the hippocampal CA1 region and increased in the hippocampal CA3 region in socially isolated LAL mice compared to control LAL mice (Fig. 3). The lack of a line difference between LAL and SAL control mice in 5-HT$_{1A}$ receptor mRNA expression in the hippocampal CA1 region, makes it difficult to judge whether control LAL mice showed an increase or isolated LAL mice showed a decrease in 5-HT$_{1A}$ receptor mRNA expression in this region. Nevertheless, social isolation induced region-specific changes in the mRNA expression of 5-HT$_{1A}$ receptors in LAL mice, whereas the same stressor had no effect on 5-HT$_{1A}$ receptor mRNA expression in SAL mice.

Thus, our data indicate that social isolation induced a higher physiological and neuroendocrine response in ‘passive’ coping LAL mice than in ‘active’ coping SAL mice. Considering these distinctly different effects of social isolation it is of interest to investigate long-lasting effects of social isolation on neuroendocrine and neurochemical parameters in these mice.
Fig. 3. Effect of social isolation for 5 days on 5-HT1A receptor mRNA expression in CA1, CA3 and dentate gyrus (DG) subregions of the hippocampus in LAL and SAL male mice. Control LAL and SAL males were housed under standard conditions with a female. A line difference was found in CA1 ($F_{(1,27)} = 11.762, P < 0.005$), CA3 ($F_{(1,27)} = 17.594, P < 0.001$) and DG ($F_{(1,27)} = 20.644, P < 0.001$), indicating lower hippocampal 5-HT1A receptor mRNA expression in LAL mice. A line × treatment interaction was found in CA1 ($F_{(1,27)} = 4.622, P < 0.05$) and CA3 ($F_{(1,27)} = 7.171, P < 0.05$), showing that 5-HT1A receptor transcript level was decreased in CA1 and increased in CA3 in socially isolated LAL mice compared to control LAL mice. * $P < 0.05$, ** $P < 0.005$, pairwise comparisons (LSD test) following Univariate ANOVA.

3.1.2. Sensory contact stress

The sensory contact stress paradigm (continuously living opposite an aggressive SAL male) consisted not only of the various stress aspects of isolation but also of the continuous psychological threat of a confrontation with an aggressive SAL male. We studied the effect of this stress paradigm for a period of 5 and 25 days and we found that this sensory contact stressor induced long-lasting changes in LAL mice whereas in SAL mice only transient stress effects were found (for overview see Table 6).

Already after one day of exposure to a SAL male, corticosterone levels were significantly higher in LAL mice than in SAL mice and this was still present after 5 and 25 days (chapter 3, 4). Body and thymic weight were decreased in both mouse lines after 5 days (chapter 4), whereas after 25 days only LAL mice
showed a reduction in body and thymic weight (chapter 3). In addition, LAL mice housed opposite a SAL male showed an increase in immobility behaviour in their home cage compared to isolated LAL mice and SAL mice housed opposite a SAL male (chapter 4), and an increase in grooming behaviour (chapter 3).

Table 6. Overview of the time-dependent effects of sensory contact stress (continuously living opposite a SAL male) for 5 and 25 days on several physiological and neuroendocrine parameters in LAL and SAL mice. The mRNA expression was measured for the MR, GR and 5-HT₁₅ receptor (5-HT₁₅ R) in the CA1 region and dentate gyrus of the hippocampus and for CRH in the hypothalamic paraventricular nucleus (PVN). Arrows indicate a significant increase (↑) or decrease (↓) compared to control LAL/SAL mice.

<table>
<thead>
<tr>
<th></th>
<th>5 days</th>
<th></th>
<th>25 days</th>
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<tbody>
<tr>
<td></td>
<td>LAL</td>
<td>SAL</td>
<td>LAL</td>
<td>SAL</td>
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<tr>
<td>Body weight</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
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<tr>
<td>Plasma Corticosterone</td>
<td>↑</td>
<td></td>
<td></td>
<td>↑</td>
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<tr>
<td>Plasma ACTH</td>
<td></td>
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<td>↑</td>
<td></td>
</tr>
<tr>
<td>Relative Adrenal weight</td>
<td>↑</td>
<td></td>
<td></td>
<td>↑</td>
</tr>
<tr>
<td>Relative Thymus weight</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>MR CA1 region</td>
<td></td>
<td></td>
<td></td>
<td>↓</td>
</tr>
<tr>
<td>Dentate gyrus</td>
<td></td>
<td></td>
<td></td>
<td>↓</td>
</tr>
<tr>
<td>GR CA1 region</td>
<td></td>
<td></td>
<td>↑</td>
<td></td>
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<tr>
<td>Dentate gyrus</td>
<td></td>
<td></td>
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<td>↑</td>
</tr>
<tr>
<td>CRH PVN</td>
<td></td>
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<td>↑</td>
<td></td>
</tr>
<tr>
<td>5-HT₁₅ R CA1 region</td>
<td></td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Dentate gyrus</td>
<td></td>
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</table>

At a central level, time-dependent changes were found in response to sensory contact stress in LAL but not in SAL mice. After 5 days, LAL mice showed an up-regulation in hypothalamic CRH and hippocampal dentate gyrus GR mRNA expression, while hippocampal MR remained unaffected (chapter 4). After 25 days, the initially up-regulated CRH and GR returned to baseline, whereas now severe down-regulation of MRs in all hippocampal subfields was detected (chapter 3). Also in other stress models time-dependent central HPA changes have been reported (Liberzon et al., 1999; Buwalda et al., 2001). These time-dependent central changes may have important implications. First, the specific pattern of central changes may have specific consequences for behavioural reactivity and stress adaptation (De Kloet et al., 1998; Korte, 2001). Second, the order of central
changes in LAL mice exposed to different durations of the sensory contact stressor can be important for understanding the development of alterations during chronic stress. It also shows that the duration of the stressor determines the pattern of stress symptoms that is observed, and, hence, the type of pathology. Third, despite the time-specific central changes in response to chronic stress, no clear time-dependent changes were found at the physiological level (i.e. elevated corticosterone levels and decreased body and thymic weights were found after 5 days but also after 25 days). Specific stress-induced changes at the central level may be more significant in understanding the effects of stress on alterations in behaviour, cognition and mood. Thus, sensory contact stress in SAL mice resulted in lower HPA reactivity during the first few days compared to LAL mice, and no long-lasting effects were found after 25 days compared to control SAL mice. This suggests successful coping by SAL mice. In contrast, the long-lasting changes in LAL mice may indicate failure of proper stress coping. To confirm the latter it would be of interest to investigating whether these deficits persist after cessation of the stressor and whether intervention with antidepressants can restore these deficits.

It was further questioned what the impact was of the type of opponent (i.e. the SAL male) in the sensory contact model on the stress response in LAL and SAL mice. It was indeed shown that the type of opponent (a SAL male opponent, a LAL male opponent or no opponent) determined the effect of sensory contact stress in LAL and SAL mice (chapter 4). While within the LAL line, exposure to a SAL male was clearly the most severe stressor (lower body and seminal vesicle weight, higher corticosterone levels and adrenal hypertrophy), within the SAL line it was exposure to a LAL male which induced most changes (relatively high levels of corticosterone, adrenal hypertrophy and elevated ACTH concentrations).

Why most changes were observed when a LAL or SAL male was housed opposite a male of the other line is unclear. It is known, however, that many animals, including mice, use odour cues to distinguish among individuals (Brown and McDonald, 1985). This ability to recognize individuals is essential to many aspects of social behaviour, such as the maintenance of stable social groups, parent-offspring or mate recognition and inbreeding avoidance. These odours are presented as chemicals on skin secretions, sexual secretions, saliva and urine conveying information such as gender, social dominance and individual identity. Some of these chemical cues are derived from interaction with the environment (such as gut bacteria or diet), whereas some others are encoded by genes. Regarding the latter, the genes of the major histocompatibility complex (MHC) may form a reliable marker of individuality due to their highly polymorphic profile. Yet, the mechanism by which MHC type influences urine odour is still unclear.

Other studies have reported a new class of proteins, termed major urinary proteins (MUP), that was found to bind and release volatile pheromones (Bachini et al., 1992; Robertson et al., 1993). In wild house mice, these MUPs have a
similar combinatorial diversity as MHC, but have a protein concentration a million times higher than MHC proteins (Beynon et al., 2001). However, like MHC proteins, the MUPs can even differ between brothers (Hurst et al., 2001a) and thus seems less likely to play a role in the recognition of a LAL or a SAL male. Interestingly, MUPs are expressed by dominant and subordinate male mice but stimulate increased scent marking only by dominant, competitive males (Hurst et al., 2001b). Urine scent marking behaviour, in particular at the partition zone in the sensory contact model, was much more pronounced in SAL males than in LAL males, irrespective of the type of their opponent (Veenema, unpublished observation). This may indicate a line difference in the perception of social dominance. Furthermore, LAL males showed significantly more immobility behaviour than SAL males when being housed opposite another male (chapter 4).

Thus, it is likely that line differences in behaviour and scent marking, probably in addition to other cues, made it possible that LAL and SAL males could recognize each other as being different. In SAL mice this also indicates that the perception of the sensory contact stressor is rather related to unfamiliarity than to the degree of aggressivity of the opponent male. The question still remains why perception of an ‘unfamiliar’ male causes a higher stress response than the perception of a ‘familiar’ type of male. Also, more research is needed to understand the invisible chemical cues that guide the natural behaviour of these so widely used laboratory animals.

3.1.3. Defeat stress

By using the sensory contact paradigm, physical contact between the two male mice was prevented during the entire experimental period. Besides this paradigm, we also investigated the effect of 21 daily short agonistic interaction (maximum of 10 min) between an aggressive SAL male and an experimental male (LAL or SAL) that were housed in triads. This physical confrontation resulted in competition for control over the territory (the cage). After establishment of a stable dominant/subordinate relationship, a perforated transparent partition separated the two males, allowing maintenance of sensory (except physical) contact. This defeat paradigm induced line-specific, long-lasting stress symptoms in both the LAL and SAL mice. It was also shown that repeated defeat was a more severe stressor than sensory contact in both mouse lines. Repeated defeat resulted for example in higher corticosterone concentrations and adrenal hypertrophy than sensory contact stress (chapter 3). At a central level defeated LAL mice showed a decrease in hippocampal MR:GR ratio whereas defeated SAL mice showed an increase in hypothalamic CRH mRNA expression compared to control LAL and SAL mice, respectively (chapter 3). Defeated LAL mice showed, however, lower body weight and higher plasma corticosterone concentrations than SAL mice (chapter 3), suggesting a higher impact of the defeat stressor in LAL mice.
Taken together, a difference in behavioural coping style as expressed by the LAL and SAL mice is associated with a differential response to repeated defeat. This may further indicate a differential susceptibility for the development of stress-related psychopathologies. Like the sensory contact paradigm, it would be of interest to investigate whether these line differences persist after cessation of the stressor and what the effect is of intervention with antidepressants.

In conclusion, by applying diverse stressors it was clearly shown that the low-aggressive LAL mice displayed a higher stressor susceptibility than the high-aggressive SAL mice. Besides stressors like forced swimming and multiple injections, also chronic stressors like social isolation, exposure to a SAL male, and repeated defeat induced more pronounced changes in LAL compared to SAL mice. This difference in stress reactivity may underly a line difference in perception of the stressor (during acute stress) as well as a line difference in stress coping or stress habituation (during chronic, or repeated stress).

3.2. Stress-induced changes in hippocampal gene expression in LAL mice (Feldker et al., 2003d).

LAL mice showed higher stressor susceptibility than SAL mice, in particular when exposed to sensory contact stress with a SAL male (chapter 3, 4). It was also found that LAL mice showed a higher expression of several cytoskeleton and signal transduction genes in the hippocampus than SAL mice (Feldker et al., 2003a,b). It was, therefore, hypothesized that chronic sensory contact stress in LAL mice would induce a down-regulation in particular in those hippocampal genes involved in plasticity. Using GeneChip analysis, it was revealed that the majority of genes differentially expressed between control LAL mice and chronically stressed LAL mice were down-regulated in the latter (71 out of 94 genes, $P < 0.01$; Feldker et al., 2003d). To our surprise, no stress-induced changes were observed in those genes that were earlier found to be higher expressed in LAL compared to SAL mice (Feldker et al., 2003a). Among these were several cytoskeleton genes, calmodulin-related genes and MAPK genes, which are likely involved in hippocampal morphology and synaptic plasticity. This strongly suggests that even under conditions of chronic stress, the cellular processes influenced by these genes are likely preserved in LAL mice.

Yet, other groups of genes were found to be affected by the chronic sensory contact stressor. Among these were several genes encoding components of the NFκB signal transduction cascade (Feldker et al., 2003d). Among these were conserved helix-loop-helix-kinase (CHUK), several ras-family members (RAB24, rap1/rap2 interacting protein, Arha2, SEK1) and NFκB responsive genes (Bcl2
binding protein homologue, Bcl2-related protein, Bcl2-associated X protein). NFκB plays a pivotal role in neuronal survival and synaptic plasticity (Mattson et al., 2000; Guo et al., 1998; Lezoualc’h et al., 1998; Maggirwar et al., 1998). In addition, it was found that NFκB activity in the hippocampus can be inhibited by glucocorticoids (Unlap and Jope, 1995), suggesting the involvement of glucocorticoids in the down-regulation of the expression of NFκB-related genes in LAL mice.

To validate the GeneChips data, in situ hybridisation was performed for three transcripts encoding CHUK, RAB24 and rap1/2 interacting protein. Moreover, an acute stress group (LAL mice exposed to sensory contact with a SAL male for 2 h) was included to be able to distinguish between acute and chronic stress effects. Interestingly, transcript level of CHUK was down-regulated in the dorsal hippocampus after chronic stress (confirming GeneChips data), whereas it was up-regulated after acute stress (Feldker et al., 2003d). This shows a clear distinction between the effects of acute and chronic stress in LAL mice. In contrast, hippocampal RAB24 mRNA was down-regulated after chronic as well as acute stress (Feldker et al., 2003d). Lastly, in situ hybridisation revealed a down-regulation in transcript level of rap1/2 interacting protein after acute but not after chronic stress (Feldker et al., 2003d). However, only the dorsal part of the hippocampus was measured, which makes it possible that the observed down-regulation of this protein after chronic stress occurred primarily in the ventral hippocampus.

These data demonstrate that sensory contact stress in LAL mice resulted in a down-regulation of several genes belonging to the NFκB signal transduction pathway. In contrast, no changes were observed in those genes that were differentially expressed between LAL and SAL mice. Future research is required to elucidate whether inhibition of NFκB signalling is involved in the observed stress symptoms in LAL mice and whether this affects hippocampal plasticity.

4. Interpretation of line differences

The data presented in this thesis showed a variety of differences between low-aggressive LAL mice and high-aggressive SAL mice on several parameters of the HPA axis, 5-HT system and hippocampal plasticity under baseline conditions and after exposure to acute and chronic stressors. The implications for these differences in terms of stress adaptation versus stress pathology are discussed in the following paragraph. Furthermore, in the last paragraph, it is discussed whether these mice can serve as an animal model to study certain aspects of human depression.
4.1. Stress adaptation versus stress pathology

In table 7 an overview is given of the LAL versus SAL differences under basal conditions and after exposure to acute and chronic stressors. In response to acute stress (forced swimming), LAL mice showed a higher magnitude of HPA changes than SAL mice. Furthermore, a higher expression of several hippocampal genes involved in morphology and neuronal plasticity was found in LAL compared to SAL mice. Interestingly, chronic stress in LAL mice did not have any effect on the expression of the genes that were differentially expressed between LAL and SAL mice. Given these data, it can be expected that LAL mice show a higher adaptive plasticity and, as a consequence, a higher flexibility than SAL mice towards stressful stimuli such as novel or changing environments. This is indeed in line with earlier studies showing clear differences in behavioural flexibility between LAL and SAL mice. In general, SAL mice react poorly to continuous changes in, for example, maze configuration, whereas LAL mice readily adjust their behaviour to new situations (Benus et al., 1987, 1988, 1990, 1991b). Interestingly, in two rat lines that differ in anxiety as well as in coping style, it was found that the cognitive performance was higher in those rats showing the ‘passive’ coping style compared to rats displaying the ‘active’ coping style (Ohl et al., 2002). Analyses of cognitive performance and emotional behaviour in two inbred strains (C57BL/6 and DBA/2) of mice further supported the idea that high anxiety or ‘passive’ coping may interact with specific cognitive processing (Ohl et al., 2003). This suggests that differential behavioural strategies as in the LAL and SAL mice, is related to differential cognitive processing. At present it is unclear whether the observed LAL versus SAL differences in HPA responsiveness and in hippocampal gene expression are related to differences in cognitive performance, but it is tempting to speculate that these two features are interrelated in these mice.

When exposed to chronic psychosocial stressors (isolation stress, sensory contact stress and defeat stress), severe and long-lasting changes in several markers of the HPA system were found in particular in LAL mice. Exposure to social isolation and sensory contact stress induced in SAL mice only a temporal stress response. In LAL mice, all stress paradigms induced a chronic increase in glucocorticoid concentrations. Elevated glucocorticoid levels may have adverse effects on brain structure and function, in particular at the level of the hippocampus (McEwen et al., 2000a,b, 2001; Sapolsky, 2000). Also, the decrease in MR:GR ratio in chronic stressed LAL mice may have contributed to further HPA aberrations (Reul et al., 1993, 2000a,b). Furthermore, LAL mice showed lower postsynaptic 5-HT1a receptor gene expression and function and lower brain 5-HT metabolism compared to SAL mice, suggesting 5-HT hypofunction in the LAL mice. Increases in synaptic 5-HT concentration are thought to mediate adaptation to stress (Chaoulloff et al., 1999). Interestingly, social status and aggressive disposition seem to depend on rapid serotonergic activation (Larson and Summers,
2001; Summers et al., 2003). This may indicate differences in behavioural responses concerning social status and stress adaptation. Finally, the rapid stress-induced suppression of hippocampal cell proliferation rate in LAL but not in SAL mice may have implications for specific functions of the hippocampus.

All together, these data may indicate that under certain conditions, LAL mice may perform better than SAL mice. However, exposure to chronic psychosocial stressors induced long-lasting changes in LAL mice which may have negative effects on the health or fitness of LAL mice. It is very difficult, however, to determine where stress adaptation ends and stress pathology begins. One approach may be to investigate whether the stress-induced changes continue to be present in LAL mice after cessation of the stressor. If so, it is likely that these mice lost their adaptive capacity and hence will develop a state of stress pathology.

**Table 7.** Overview of the LAL versus SAL differences in several parameters of the HPA system, 5-HT system and hippocampus under basal, acute stress and chronic stress conditions. Arrows indicate an increase or decrease compared to SAL mice, n.m. means not measured. No arrow indicates that there is no line difference.

<table>
<thead>
<tr>
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<th>LAL : SAL</th>
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<tbody>
<tr>
<td></td>
<td>Basal</td>
</tr>
<tr>
<td><strong>HPA system</strong></td>
<td></td>
</tr>
<tr>
<td>ACTH</td>
<td>↓</td>
</tr>
<tr>
<td>Corticosterone</td>
<td></td>
</tr>
<tr>
<td>less fluctuation</td>
<td></td>
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<tr>
<td>MR:GR</td>
<td></td>
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<tr>
<td>CRH</td>
<td></td>
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<tr>
<td><strong>5-HT system</strong></td>
<td></td>
</tr>
<tr>
<td>5-HT₁A function*</td>
<td>↓</td>
</tr>
<tr>
<td>5-HT turnover</td>
<td>↓</td>
</tr>
<tr>
<td><strong>Hippocampal gene expression</strong></td>
<td></td>
</tr>
<tr>
<td>Cytoskeleton genes</td>
<td>↑↑</td>
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<tr>
<td>Signal transduction genes</td>
<td>↑↑ #</td>
</tr>
<tr>
<td>Hippocampal cell proliferation</td>
<td>↓↓</td>
</tr>
</tbody>
</table>

* only postsynaptic, also based on Van der Vegt et al. (2001), Van Riel et al. (2002); # only in LAL versus control LAL mice
4.2. Animal model for depression?

The objective of this thesis was to find causal factors underlying individual differences in susceptibility to stress and stress-related psychopathologies. This was studied in two lines of mice that were subjected to different stress paradigms. The interest for these particular selection lines of mice was based on the line differences found for plasma corticosterone levels and 5-HT$_{1A}$ receptor gene expression, which are markers of two systems (HPA and 5-HT systems) that are closely related to depression. The interest for stress models is based on the association between stress and depression. It is suggested that chronic stress precipitates depression by long-lasting activation of stress systems that eventually will result in dysregulation of neuroendocrine and neurochemical systems. There are at least three observations that fit with this assumption. First, HPA abnormalities are found in about half of the patients suffering from severe depression (Rubin et al., 1987; Gold et al., 1988; Holsboer and Barden, 1996; Nemeroff, 1996). Second, excessive exogenous or endogenous circulations of glucocorticoids have a tremendous influence on depressive mood (Krystal et al., 1990; Murphy, 1991; Wolkowitz et al., 1992; Reus and Wolkowitz, 2001). Third, there is evidence that losses or other negative life events precede the first episode of depression (Brown et al., 1994; Frank et al., 1996; Kohn et al., 2001).

We characterized the LAL and SAL mice for their neuroendocrine and neurochemical regulation under basal, acute and chronic stress situations. To function as a potential animal model for depression, LAL and SAL mice should fulfil three criteria (Willner, 1984):

1. **Construct validity:** the model mimics the etiology of depression
2. **Face validity:** the model replicates a number of symptoms found in human depression
3. **Predictive validity:** treatment of symptoms brings about identical effects as in humans

With regard to the first criterion, LAL and SAL mice were exposed to chronic psychosocial stressors, which are often the principal stressors for humans. Concerning the second criterion, under basal conditions and after exposure to stress, LAL mice showed some characteristics compared to SAL mice, which may be considered as potential risk factors to develop a state of depression. The third criterion has not been tested in the LAL and SAL mice. The LAL versus SAL differences concerning the second criterion are discussed in the following paragraphs.

Under baseline conditions, LAL and SAL mice differed in some markers of the HPA and 5-HT systems, hippocampal plasticity and behaviour, which may be considered as a line difference in the predisposition for stress-related
psychopathologies like depression. First, LAL mice showed no fluctuation in plasma corticosterone concentrations around the circadian peak. A less pronounced circadian rhythm is also found in patients suffering from depression (Deuschle et al., 1997; Weber et al., 2000). Second, LAL mice showed clear signs of central 5-HT hypofunction under baseline conditions and in response to an acute stressor, and a reduced gene expression and function of postsynaptic 5-HT\textsubscript{1A} receptors. Abnormalities in the 5-HT system also occur in patients suffering from depression (Owens and Nemeroff, 1994; Baldwin and Rudge, 1995; Maes and Meltzer, 1995). Markers of the serotonergic system were found to be reduced in the brains of depressed suicide victims (Mann et al., 2000). Furthermore, decreases in 5-HT\textsubscript{1A} receptor function and binding have been found in depression (Lopez et al., 1998; Drevets et al., 2000). Third, LAL mice showed a fast suppression of hippocampal cell proliferation rate. Cell proliferation or neurogenesis is a form of structural remodelling of the hippocampus. This remodelling of the hippocampus is likely to be affected by depression as well (McEwen, 2000a,b; Sapolsky, 2000, 2001). Finally, LAL and SAL mice differ in their response initiation to stimuli. LAL mice show high immobility behaviour in, for example, the forced swim test. Interestingly, the active state of patients suffering from depression is also decreased (Ressler and Nemeroff, 2000). Taken together, these line differences may indicate that LAL mice are more susceptible to develop stress-induced depression. However, it can also be argued that these line differences belong to a general variety in behavioural, neuroendocrine and neurochemical responses to environmental stimuli.

Exposure to chronic psychosocial stressors induced changes, in particular, in the LAL mice, that may mimic some symptoms found in human depression. First, exposure to chronic psychosocial stressors induced a long-lasting increase in plasma corticosterone levels. Human depression is often associated with hypercortisolism (Rubin et al., 1987; Gold et al., 1988; Holsboer and Barden, 1996; Nemeroff, 1996). In addition, diseases in which there is an excessive concentration of glucocorticoids are often correlated with depressive mood (Krystal et al., 1990; Murphy, 1991; Wolkowitz et al., 1992; Reus and Wolkowitz, 2001). Second, changes were found in the hippocampal MR:GR ratio in LAL mice after sensory contact stress and repeated defeat stress. Reduced levels of, in particular, hippocampal MRs have been hypothesized to be involved in the HPA aberrations seen in human depression (Lopez et al., 1998; Reul et al., 1993, 2000a,b). Accordingly, by exposing LAL mice to chronic psychosocial stress certain neuroendocrine features found in human depression can be mimicked.

However, we did, for instance, not find long-lasting stress-induced changes in 5-HT\textsubscript{1A} receptor gene expression, which is frequently observed in rodents exposed to chronic stress, but also in suicide victims with a history of depression (Lopez et al., 1998). In addition, rather small differences in behaviour were found in chronically stressed LAL or SAL mice. Several other studies have shown that
psychosocial stress induced clear behavioural alterations generally associated with increased anxiogenic-like behaviour and decreased exploration and/or locomotor activity (Keeney and Hogg, 1999; Berton et al., 1998). However, one could argue that under basal conditions, LAL mice already show a lower 5-HT$_{1A}$ receptor gene expression, and a behavioural trait associated with high immobility compared to SAL mice. Clearly more experiments are needed, which include the Dex/CRH test, and an experiment to investigate whether the stress-induced symptoms in LAL mice continue to be present after cessation of the stressor.

At present, we know too little to state whether the stress-induced changes observed in the LAL mice can be considered as stress adaptation or stress pathology. However, the profound line differences found for HPA regulation and 5-HT functioning, as well as behaviour and hippocampal plasticity, indicate that the LAL and SAL mice are a relevant and interesting animal model, not only to study differences in stress coping, but also to study in LAL mice the development of some depression-like symptoms under conditions of chronic stress.

5. Concluding remarks

Based on the observations presented in this thesis it is concluded that a difference in behavioural coping style, as displayed by the low-aggressive LAL mice and the high-aggressive SAL mice, is paralleled by differences in HPA regulation, 5-HT functioning and hippocampal plasticity. The differences found under baseline or acute stress conditions (higher HPA responsivity, lower 5-HT functioning, higher expression of specific hippocampal genes and lower hippocampal cell proliferation rate in LAL compared to SAL mice), suggests a difference in the predisposition to stress-related mood disorders, like depression. The long-lasting increased activity of the HPA axis in response to chronic stress in LAL compared to SAL mice, as well as the changes in hippocampal MR:GR balance and changes in hippocampal genes belonging to the NFκB signal transduction pathway in LAL mice, reflect clear differences in stress coping and may point towards a stress-induced pathogenesis in LAL mice. In particular the LAL mice are, therefore, of interest to study certain aspects of stress-related depressive behaviour. In conclusion, this thesis provides evidence that a considerable variation exist between two behavioural coping styles in the neuroendocrinological and neurochemical consequences of stress and the susceptibility to stress-related psychopathologies. Individual differences, represented by the LAL and SAL mice, may be the key in the search for factors that determine a differential susceptibility to stress-related mood disorders.
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Chapter 7


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