Chapter 1

GENERAL INTRODUCTION
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1. Introduction

Life requires organisms to adapt to changing environmental demands. These emotionally and/or physically adaptations are in general referred to as the stress response, and involve behavioural, neuroendocrine and neurochemical changes. Failure of proper adaptation to the stressor will result in sustained over-activation of several stress systems and this may lead to the development of stress-related psychopathologies such as anxiety and depression. The way animals, including humans, cope with stressful events shows, however, a considerable individual variation, which may underlie a differential vulnerability to the development of stress-related pathologies. Why some individuals are more likely to suffer from stress-related pathologies than others under seemingly similar conditions is still an unresolved phenomenon.

This thesis focused on further analysis of individual differences in stress reactivity. The objective was to find causal factors underlying individual differences in susceptibility to stress and stress-related psychopathologies. These individual differences will be studied in two lines of mice, that show distinctly different behavioural strategies towards environmental stimuli. It is hypothesized that this difference in behavioural coping style has, as consequence, a differential susceptibility to stressors. The focus will be on two systems involved in stress adaptation: i.e. the hypothalamic-pituitary-adrenal (HPA) axis and the serotonergic (5-hydroxytryptamine, 5-HT) system. In the following paragraphs the role of the HPA axis, the 5-HT system in stress and stress-related psychopathologies will be introduced. In addition, the current knowledge of the selection lines of mice will be reviewed.

2. The stress response

Stress is defined as a state of threatened homeostasis, which can be of physical and/or psychological nature. In particular psychological and social stimuli were found to be powerful ‘stressors’ (Mason, 1971; Koolhaas et al., 1997). The stress response is an adaptive compensatory response of the organism to sustain homeostasis. The concept of stress was first described by Selye (1936, 1950). He was intrigued by the non-specificity of the bodies response to stress. Indeed, any perceived stressor will lead to the activation of the autonomic nervous system and of the Hypothalamus-Pituitary-Adrenal (HPA) axis. However, the characteristics of the stressor (such as type, duration, predictability and controllability of the stressor), the effects of the endproducts of the stress response and individual differences (genetical factors and experience) results in very specific effects of the stress response. Identifying individual differences in stress responses in addition to
stressor-specific pathways in brain may be essential for understanding and treatment of the pathogenesis of stress-related disorders.

In response to stress, activation of the autonomic nervous system and the HPA axis will subsequently lead to behavioural, physiological and neurobiological adaptations. Initially the stress response will lead to an increase in sympathetic nervous activity. This sympathetic activity results in an increased release of adrenaline (from the adrenal medulla) and noradrenaline (from other sympathetic nerve endings throughout the body) into the bloodstream. These catecholamines stimulate the heart and increase the blood flow to the central nervous system and muscles within seconds, allowing the organism to directly respond to the stressor by vigilance, arousal, and activation. Activation of the HPA axis results in the secretion of glucocorticoid hormones. Peak levels of these hormones are usually achieved 10 to 30 min after the onset of the stress response. The primary action of the glucocorticoids is to elevate blood glucose levels. In addition, glucocorticoids are very important to suppress the stress response, to mediate recovery from the stress response and to prepare for the next encounter. Both the neuroendocrine and autonomic systems interact at several levels, and proper functioning of these systems is essential for normal adaptation to stress.

In most cases, a brief period of controllable stress can be experienced as excitement and may be beneficial to emotion and health. If, however, the stressor is chronic or uncontrollable, the HPA axis is activated for a prolonged time period. This can lead to a state of hypercorticism and disturbed negative feedback function. In particular, overexposure to corticosteroids may result in changes in mood and cognition by affecting neurotransmitter systems such as the serotonergic (5-HT) system. In humans, stress is regarded as an important risk factor for the development of depression. Depression is often associated with disturbances in 5-HT system and HPA system. Therefore, this thesis will primarily focus on functioning of the HPA axis and 5-HT system during stress and their possible role in stress-related mood disorders. The interaction between stress, the HPA axis and the 5-HT system as well as stress-related psychopathologies and in particular depression is explained in the following paragraphs.

2.1. The HPA axis

2.1.1. Description and function of the HPA axis

The HPA axis is activated during stress but it also coordinates circadian events, such as food intake and the sleep/wake cycle. This diurnal activity of the HPA axis results in a peak of glucocorticoid hormone secretion at the onset of the active period (which is the dark period in night active animals). When the HPA axis is
activated during stress or around the circadian peak, corticotropin-releasing hormone (CRH) is produced in neurons within the paraventricular nucleus (PVN) of the hypothalamus. CRH stimulates the anterior pituitary to release adrenocorticotropic hormone (ACTH) into the bloodstream. In the adrenal cortex, ACTH is bound to its receptors and this stimulates the synthesis of glucocorticoids (cortisol in humans, corticosterone in rodents) from cholesterol and increases the subsequent secretion of these steroids into the systemic circulation (Fig. 1).

Fig. 1.
Schematic representation of the hypothalamus-pituitary-adrenal (HPA) axis. Bold arrows indicate the hormonal cascade that triggers the release of corticosterone from the adrenal glands. Dashed arrows represent the feedback actions of corticosterone on the level of the pituitary and PVN which involves GR activation (negative feedback) and at the level of the hippocampus (negative and/or positive feedback) which can be a synergistic activity of colocalized MRs and GRs in addition to specific MR and GR effects.

For many years, it was believed that these glucocorticoid hormones mainly acted at the level of the peripheral nervous system via binding to intracellular glucocorticoid receptors (GRs) which are found in most peripheral organs. However, this idea was completely changed since the discovery of receptors for stress hormones within the brain (McEwen et al., 1968). Glucocorticoids easily enter the brain due to their lipophilic nature. Here they can bind to mineralocorticoid receptors (MRs) in addition to GRs (De Kloet and Reul, 1987). MRs are also present in the kidney regulating the salt and water balance of the organism by binding aldosterone rather than glucocorticoid hormone. In contrast with the brain, glucocorticoids in the kidney are metabolically inactivated by the presence of 11beta-hydroxysteroid dehydrogenase (11β-HSD) type 2.
The MR and GR differ in their distribution in the brain and in their affinity for corticosteroids. The MR is restricted to ‘limbic’ brain structures (hippocampus, amygdala and septum), whereas the GR has a widespread distribution, and is abundantly expressed in brain regions involved in the stress response such as the hypothalamus, hippocampus, amygdala, various brain stem nuclei and pituitary (Van Eekelen, 1988; Chao, 1989). MRs have a high affinity for corticosterone, approximately 10-fold higher than GRs (Reul and De Kloet, 1985). As a consequence, MRs are predominantly occupied at low corticosterone levels (during basal trough), whereas additionally GRs will become occupied when corticosterone levels are high around the circadian peak and during stress (Reul and De Kloet, 1985, Reul et al., 1987). This differential affinity for corticosterone in addition to the differential distribution of the two receptors may indicate that corticosterone has differential functions after binding to the MR or GR. MRs determine the sensitivity of the HPA response and are thought to be involved in evaluating of environmental stimuli, preparing the organism for a certain behavioural response in order to limit homeostatic disturbance as much as possible (De Kloet et al., 1993, 1998). Activation of GRs (always in addition to already activated MRs) is needed for the termination of the HPA response, to mediate recovery from stress, to facilitate behavioural adaptation, and to prepare to following stressors (De Kloet et al., 1993, 1998).

2.1.2. Mechanism of corticosteroid action

When corticosteroid hormones enter the brain they can pass the cell membrane and bind to MRs and GRs localized in the cytoplasm. These steroid-receptor complexes are then translocated from the cytosol to the nucleus where they bind as dimers to specific DNA sequences and act as transcription factors. This results in enhancement or repression of gene transcription and thus in alterations in protein synthesis. The highest density of MRs and GRs is found in the hippocampus, where these receptors are colocalized and can form heterodimers in addition to homodimers (Trapp, 1994). The hippocampus (see Fig. 2 for anatomical structure) is involved in functions such as mood, cognition and behaviour (Isaacson, 1974). Thus both the MR and GR in the hippocampus may play a critical role in these functions as well. Indeed, a variety of research findings confirmed the impact of hippocampal MR and GR actions on neuroendocrine regulation (Jacobson and Sapolsky, 1991; Dallman et al., 1992), neuronal function (Joëls and de Kloet, 1992a, 1994), neuronal plasticity (McEwen, 1994; Magarinos, 1996), cognition (Oitzl and de Kloet, 1992; De Kloet et al., 1999) and recently also hippocampal neurogenesis (Gould et al., 1997; Gass et al., 2000). Thus, actions mediated by MR and GR in hippocampal neurons appear to be critical for neuronal functioning. This has led to the hypothesis that a “balance in hippocampal MR- and GR-mediated
effects exerted by corticosteroids is critical for homeostatic control” (De Kloet, 1991; De Kloet et al., 1998).

To prevent the HPA axis from overshooting, negative feedback actions are exerted via MR and GR activation at various levels within the HPA axis. In response to high levels of glucocorticoids (around the circadian peak and during stress) synergistic activation of both MRs and GRs leads to a suppression of the HPA axis (Ratka et al., 1989; Bradbury et al., 1994). At the hypothalamic and pituitary level, GR occupation leads to inhibition of HPA activity by suppressing the transcripts of genes encoding CRH and ACTH, respectively (De Kloet and Reul, 1987; Meaney et al., 1991; De Kloet et al., 1998). At the level of the hippocampus, MR activation exerts a tonic inhibitory control on the HPA axis when corticosteroid levels are low (Dallman et al., 1989; Ratka et al., 1989).

![Diagram of the hippocampus](image)

**Fig. 2.** Schematic coronal representation of the dorsal part of the hippocampus. The hippocampal formation comprises four regions: dentate gyrus (DG), Ammon’s horn (CA1, CA2, CA3, CA4), subiculum, entorhinal cortex. The entorhinal cortex receives information from many brain regions and projects onto granule cells within the dentate gyrus, which on their turn project via the mossy fibres onto the pyramidal cells in the CA3 region. These pyramidal cells project via Schaffer collaterals onto CA1 pyramidal cells, which in turn project back to the entorhinal cortex via the subiculum.

### 2.1.3. HPA dysregulation in depression

Stress is a major factor that has been thought to be involved in mood disorders such as anxiety and depression. Stressful life events often trigger the onset of depressive episodes. Depression is one of the most prominent stress-related
psychiatric disorders, affecting the lives of approximately 10-15% of the population, and leading to great personal and social costs. Although the precise mechanism by which stress precipitates depression is not clear, the HPA axis and the 5-HT system seem to be involved. The abnormalities in the HPA axis of depressed patients together with the delay in therapeutic effects of antidepressants have led to the hypothesis that antidepressants may act primarily through normalisation of the HPA system (for review see Holsboer and Barden, 1996).

Abnormalities in the HPA axis, seen in about half of the patients suffering from major depression, include increased concentrations of cortisol in plasma, urine, and cerebrospinal fluid (CSF), an increased cortisol response to ACTH, and enlargements of pituitary and adrenal glands (Gold et al., 1988; Owens and Nemeroff, 1993; Holsboer and Barden, 1996; Nemeroff, 1996). In addition, an increase in CRH in the CSF, increased CRH mRNA and protein in the PVN, and a blunted ACTH response to CRH have been reported in depressed patients (Gold et al., 1988; Nemeroff, 1996). Finally, depressed patients showed non-suppression of cortisol and ACTH secretion following administration of the synthetic glucocorticoid dexamethasone (dexamethasone suppression test) or in response to CRH following dexamethasone pretreatment (Dex/CRH test) (Owens and Nemeroff, 1993; Heuser et al., 1994; Holsboer and Barden, 1996; Nemeroff, 1996). This latter phenomenon is thought to be induced by diminished functioning of the corticosteroid receptors in brain and pituitary (De Kloet et al., 1998; Holsboer, 2000; Reul et al., 2000). Thus, depressed patients show hypercortisolism, increased CRH activity, and impaired glucocorticoid-mediated feedback inhibition.

These HPA alterations are thought to play a causal role in the pathogenesis of depression. Successful antidepressant treatment of patients with major depression was found to be associated with normalization of HPA axis activity (Linkowski et al., 1987; Wodarz et al., 1992; Heuser et al., 1996; Deuschle et al., 1997). This effect may have been mediated by an increase in corticosteroid receptor function. Indeed, an up-regulation of GR mRNA and protein was found in several animal studies after long-term treatment with antidepressants (Brady et al., 1992; Pepin et al., 1992a; Seckl and Fink, 1992; Przegalinski and Budziszewska, 1993; Reul et al., 1993; Peeters et al., 1994; Rossby et al., 1995). Chronic antidepressant treatment was also found to attenuate several endocrine (elevated levels of corticosterone and ACTH) and behavioural abnormalities (cognitive impairment) in transgenic mice with a reduced GR expression (Pepin et al., 1992b; Montkowski et al., 1995). In addition to GR, MR dysregulation may also contribute to the increased HPA activity found in depressed patients (Reul et al., 2000). Reduced levels of MR were found in the hippocampi of suicide victims (Lopez et al., 1998). Furthermore, MR antagonist studies revealed that a decrease in hippocampal MR functioning leads to increased activity of the HPA axis (Oitzl et al., 1994; Heuser et al., 2000). In
addition, rats that were chronically stressed showed a decrease in MR gene expression, which could be prevented by treatment with the tricyclic antidepressant desipramine (Lopez et al., 1998). Other studies showed an up-regulation of the MR after chronic treatment with antidepressants (Reul et al., 1993, 1994; Seckl and Fink, 1992; Yau et al., 1995). Thus, a balance in MR- and GR-mediated effects on the HPA system is of crucial importance to restrain HPA activity (De Kloet et al., 1998).

As the time course of antidepressant actions on corticosteroid receptors coincide with normalization of HPA activity, and follows closely clinical improvement of depression (Reul et al., 1993, 1994), it was hypothesized that antidepressants may elevate mood in depressed patients through their effects on corticosteroid receptors (Barden et al., 1995). The primary action of antidepressants could be stimulation of corticosteroid receptor gene expression and thereby normalization of the HPA system (Barden et al., 1995). The mechanism by which corticosteroids affect mood in depression is, however, not well understood, although it is likely that this involves an interaction between corticosteroids and neurotransmitter systems such as the 5-HT system.

How the HPA alterations observed in human depression are being developed is far from clear. A complex interaction between genetic background and environmental factors are thought to determine the susceptibility for depression. In particular alterations in the GR gene or GR-regulated genes are thought to contribute to a genetic susceptibility for depression. In this light, the data from the Munich Vulnerability Study are of interest as they show that about 20% of the subjects who never suffered from a psychiatric disorder, but belong to families with high genetic load for depression display abnormal responses to the Dex/CRH test, suggesting impaired corticosteroid receptor functioning (Holsboer et al., 1995; Modell et al., 1998). Recently, it has been proposed that a hyperactive CRH system in addition to a dysfunctioning of corticosteroid receptors may underly the HPA abnormalities seen in depressed patients (Reul and Holsboer, 2002). Hypersecretion of glucocorticoids, induced by impaired glucocorticoid feedback inhibition, may lead to an increase in the synthesis of CRH in the central amygdala (CRH in this region is under positive control of glucocorticoids [Schulkin et al., 1998]). This may result in enhanced input from the central amygdala to the PVN, where it has a stimulatory influence on the HPA axis (Reul et al., 2000). In such a way, a positive feed-forward loop may develop between the amygdala and the PVN, resulting in a sustained hyperactivity of the HPA axis (Reul and Holsboer, 2002).

The recently discovered endogenous ligands besides CRH, which are urocortin I, II, and III (Vaughan et al., 1995; Bittencourt et al., 1999; Reyes et al., 2001; Hsu and Hsueh, 2001; Lewis et al., 2001), and the presence of two types of CRH
receptors (Chalmers et al., 1995), may give new insights into the involvement of the
CRH family in the stress response and in stress-related psychopathologies (see
Reul and Holsboer, 2002). Activation of the type 1 CRH receptor by CRH, is
essential for the acute phase of the stress response, whereas activation of the type 2
CRH receptor by urocortin II and III, mediates the stress-coping or stress-recovery
responses (Hsu and Hsueh, 2001). Although more knowledge is required, both the
CRH system and the corticosteroid receptors are interesting targets for the
development of novel antidepressant drugs.

The question still remains when corticosteroid hormones, that normally ensure
survival during a period of stress, become damaging to health. In addition, it is
unclear why stressful life events may precipitate a depressive episode in some
subjects and not in others. It is likely that a complex interplay between genetic
susceptibility and environmental conditions determine the risk of developing
depression or other stress-related disorders. Understanding the individual
differences in adaptive responses to stress is the topic of this thesis and may help to
find clues in the relationship between stress and depressive illness.

2.2. The brain serotonin system

2.2.1. Description and function of the serotonin system

Serotonin (5-hydroxytryptamine or 5-HT) is a bioamine synthesized from the
amino acid tryptophan and acts as a neurotransmitter in the brain. The cell bodies
containing 5-HT are found in nine clusters located in midbrain, pons and medulla.
Two clusters are the dorsal raphe and medial raphe nuclei (DRN and MRN,
respectively) located in the midbrain, which have axonal projections to the
forebrain, although they appear to have different functions. The widespread
anatomical distribution of serotonergic neurons throughout the brain is shown in
Figure 3.

Serotonin produces its effects through a high variety of membrane bound
receptors (up till now there are 15 different types discovered). All 5-HT receptors
(in classes of 5-HT1 through 5-HT7) are G-protein-coupled receptors, except for
the 5-HT3 receptor which is a ligand-gated ion channel. The 5-HT1 receptor
family contains five receptor subtypes, of which the 5-HT1A receptors are widely
distributed throughout the brain. In the raphe nuclei, the 5-HT1A receptors are
somatodendritic, and act as autoreceptors to inhibit cell firing of raphe serotonergic
cells. Postsynaptic 5-HT1A receptors can be found in a number of limbic structures.
The 5-HT1A receptors are linked to G-protein cAMP second messenger systems
which regulates K⁺ channels. Activation of 5-HT1A receptors causes neuronal
hyperpolarization, leading in general to suppressed activity of the neurons.
2.2.2. Serotonin dysregulation in depression

5-HT facilitates adaptation to stress. Disturbances in 5-HT functioning have been linked to several psychiatric disorders such as depression, anxiety, aggression and impulse control (Owens and Nemeroff, 1994; Baldwin and Rudge, 1995; Maes and Meltzer, 1995; Kavoussi et al., 1997; Van Praag, 1998). Effective clinical treatment of anxiety and depression has been accomplished with drugs that act to potentiate monoaminergic neurotransmission (Baker and Greenshaw, 1988). Although the first generation of antidepressant drugs (tricyclic and monoamine oxidase inhibitors), was discovered by serendipity in 1950s, the new ones (such as selective serotonin reuptake inhibitors) were developed with the idea that monoaminergic deficiency is a central component of affective disorders. In particular 5-HT hypofunction seem to be related to human depression. Markers for the serotonergic system were reduced in the brain of depressed suicide victims (Mann et al., 2000). In addition, $5\text{-HT}_{1A}$ receptors are considered as a relevant target for the treatment of anxiety and depression (Lesch, 1991; De Vry, 1995). Selective $5\text{-HT}_{1A}$ receptor agonists, such as buspirone and gepirone, were found to be effective in the treatment of anxiety and depression in humans (Rickels and

Fig. 3. Schematic midsagittal section of the rodent brain, showing the locations of the dorsal and median raphe nuclei in the midbrain (brown ovals), and the widespread distribution of the axons of the serotonergic neurons throughout the entire brain (Adapted from Consolazione and Cuello, 1982).
Schweitzer, 1990; Fabre, 1990; Robinson et al., 1990; Den Boer et al., 2000). This role of 5-HT<sub>1A</sub> receptor in anxiety and depression was recently supported by studies utilising 5-HT<sub>1A</sub> receptor knockout mice. These mice showed an increase in anxiety- and depression-related behaviours (Heisler et al., 1998; Parks et al., 1998). During the past decade it is, however, questioned whether 5-HT deficiency is a cause or a consequence of depression. The time course of antidepressant action in addition to HPA alterations seen in depressed patients have led to the hypothesis that antidepressants might elevate mood by primarily acting on the HPA axis. Although the precise mechanism of antidepressant action is still unclear, it is known that the HPA axis and the 5-HT system interact at numerous levels, and that proper functioning of both systems is required for stress adaptation.

2.3. Interactions between the HPA and serotonin systems

The serotonergic system can interact with the HPA axis in a bidirectional way. Serotonin can have effects on several components of the HPA axis. Serotonergic neurons projecting from the raphe nuclei form synapses with CRH-containing neurons in the PVN (Fuller, 1990) and can thereby influence the release of CRH, ACTH and corticosterone (Fuller, 1990; Fuller and Snoddy, 1990; Van de Kar 1991). Furthermore, the 5-HT<sub>1A</sub> receptor appears to mediate activation of the HPA axis (Fuller 1990; Jorgensen et al., 2002).

Serotonergic neurotransmission can also be modulated by corticosteroids. The stress-induced 5-HT synthesis rate can be reduced by removal of the adrenal glands (adrenalectomy), while corticosterone replacement normalizes this 5-HT response (De Kloet et al., 1982; Singh et al., 1990; Korte-Bouws et al., 1996). This effect of corticosterone is probably mediated by GR activation in the hippocampus which is projecting to the raphe nuclei (Singh et al., 1990; Linthorst et al., 1995). In particular in the hippocampus, interactions occur between the 5-HT<sub>1A</sub> receptor (the most abundant serotonin receptor in this region) and colocalized MR and GR (Joëls et al., 1991). Adrenalectomy induced an increase in hippocampal 5-HT<sub>1A</sub> receptor gene expression in particular in the dentate gyrus (De Kloet et al., 1986; Chalmers et al., 1993), which could be prevented by administration of low levels of corticosterone (Chalmers et al., 1993). Additionally, electrophysiological studies have shown that 5-HT-induced hyperpolarization in CA1 and dentate gyrus hippocampal neurons was suppressed by predominant activation of MRs (Joëls et al., 1991). Thus, hippocampal 5-HT<sub>1A</sub> receptor expression and functioning is under tonic inhibition of corticosterone when the hormone predominantly activates MRs (Meijer and de Kloet, 1995).

By contrast, higher concentrations of corticosterone resulting in combined occupation of MR and GR were found to overrule the MR effects and induced a large 5-HT<sub>1A</sub> receptor-mediated hyperpolarization (Joëls and de Kloet, 1992b). When corticosterone levels are chronically elevated, either pharmacologically or
due to chronic stress, hippocampal 5-HT$_{1A}$ receptor binding and gene expression was down-regulated (Watanabe et al., 1993; Meijer et al., 1997; Lopez et al., 1998; Veenema, unpublished observation) and 5-HT$_{1A}$ receptor responsiveness was decreased (Karten et al., 1999). This effect may have been mediated by over-activation of MRs (Meijer et al., 1997). Meijer and colleagues (1997) showed that flattening of the diurnal corticosterone rhythm (by elevated basal trough levels), resulting in predominant MR activation, induced a similar decrease in hippocampal 5-HT$_{1A}$ receptor expression and binding. Corticosteroids had no effect on presynaptic 5-HT$_{1A}$ receptor gene expression in the dorsal raphe nucleus (Chalmers et al., 1993). This suggests that the activity of the hippocampal 5-HT system can be modulated by MR (tonic inhibition) and GR (facilitation) and that a balanced activation between MR and GR is important for normal 5-HT$_{1A}$ receptor function.

3. Individual susceptibility to stressors

The way individuals cope with stressful situations shows a high variability in behavioural responses. Extensive research has shown that at least two alternative behavioural strategies to cope with environmental stimuli can be distinguished in several species including mice, rats, birds, pigs, monkeys and humans (Benus et al., 1991a; Crusio et al., 1991; Hinton et al., 1991; Ursin et al., 1993; Koolhaas et al., 1999). One is the ‘active’ coping style (or fight-flight) and the other is the ‘passive’ coping style (or conservation-withdrawal). These alternative behavioural strategies are accompanied by differences in neuroendocrine and physiological responses. Active coping is associated with higher sympatho-adrenal activity and lower HPA reactivity, whereas passive coping is characterised by predominant parasympathetic activity and higher HPA reactivity (Bohus et al., 1987, 1993; Koolhaas, 1994; Sgiofo et al., 1996; Korte et al., 1997, 1999). Both coping styles have a distinct adaptive value and may be important for the survival of the species by regulation of the population. This is supported by the finding that in wildlife rodent populations, both the active and passive behavioural coping styles are encountered at a higher rate than expected by chance (Bohus et al., 1987; Koolhaas et al., 1999). The main difference between the two coping strategies is likely the degree of behavioural plasticity or flexibility (Koolhaas et al., 2001). This may have implications for a differential susceptibility for stress-related diseases. Two selected lines of wild house mice were used as animal model to test for individual differences in stressor susceptibility. This thesis will focus on differences in baseline and stress-induced activity and functioning of the HPA and 5-HT systems in the two mouse lines that show distinctly different behavioural coping strategies. These selection lines will be introduced in the next paragraph.
3.1. Genetically selected mouse lines

Male mice, originating from a colony of wild house-mice (*Mus musculus domesticus*) maintained at the University of Groningen, The Netherlands, have been selected for long attack latency (LAL) and short attack latency (SAL). The original colony descended from four males and three females caught from the wild in 1971. Until 1973, the mice were bred at random after which selection for attack latency started with 21 males and 21 females (see Fig. 4). All young adult males (92-100 days of age) are tested for their attack latency (Van Oortmerssen and Bakker, 1981). In this test, LAL and SAL males are confronted with a standard non-aggressive opponent male of an inbred albino strain (MAS-Gro) at the border of their home cage. The time it takes before a LAL or SAL mouse attacks the non-aggressive opponent (with a maximum duration of 600 s) is measured on three consecutive days. The attack latency score is the mean of these daily scores.

![Generations table](image)

**Fig. 4.** Genetic selection for long attack latency (LAL, dashed line) and short attack latency (SAL, solid line) in males of wild house-mice. Several attempts were necessary to successfully breed low-aggressive LAL males. At present, SAL males have an attack latency score of less than 50 s, and most LAL males do not attack within the experimental time (which is $3 \times 600$ s).
Besides their differences in attack latency, the LAL and SAL mice show profound differences in their behavioural response when exposed to environmental challenges (see Table 1). For example, LAL mice suppress their activity when exposed to uncontrollable or unescapable stressors (Sluyter et al., 1996), but perform very well in a changing environment (Benus et al., 1987). SAL mice perform better in a two-way shock avoidance (Benus et al., 1989), but show more routine-like behaviour in both social and non-social situations (Benus et al., 1988, 1990). Thus, LAL mice seem to depend more on environmental cues whereas SAL mice are more intrinsically controlled. Furthermore, SAL and LAL mice differ in their response initiation to stressors. LAL mice seem to respond only when absolutely necessary, and show a high degree of behavioural flexibility, whereas SAL mice respond immediately to a challenge by showing active behaviour. All together, it was concluded that LAL males display a ‘passive’ or ‘reactive’ coping style when exposed to new environmental stimuli whereas SAL males show an ‘active’ or ‘proactive’ coping style (Benus et al., 1991a).

Table 1. Overview of the behavioural differences between low-aggressive LAL males and high-aggressive SAL males.

<table>
<thead>
<tr>
<th>Behavioural characteristics</th>
<th>LAL</th>
<th>SAL</th>
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<tbody>
<tr>
<td>Attack latency</td>
<td>Long</td>
<td>Short</td>
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<tr>
<td>Active Avoidance</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Defensive burying</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Nest-building</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Routine formation</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Day-night reversal</td>
<td>Fast</td>
<td>Slow</td>
</tr>
<tr>
<td>Cue dependency</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Conditioned immobility</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Flexibility</td>
<td>High</td>
<td>Low</td>
</tr>
</tbody>
</table>

These behavioural differences in LAL and SAL mice were each associated with a specific pattern of neuroendocrine and neurochemical responses (see Table 2). Hippocampal 5-HT\textsubscript{1A} receptor transcript and binding was found to be lower in LAL mice, which was associated with higher plasma levels of corticosterone (Korte et al., 1996). As compared to SAL mice, LAL mice had larger hippocampal mossy fibre terminal fields (Sluyter et al., 1994), which is thought to be positively related to spatial learning (Prior et al., 1997). During the neonatal period, LAL mice are exposed to higher levels of testosterone than SAL mice (Compaan et al.,
1992; De Ruiter et al., 1992). This may be related to a differential organisation of the testosterone dependent vasopressin system. Indeed it was found that the density of vasopressinergic (AVP) neurons in the bed nucleus of the stria terminalis and its innervation of the lateral septum was higher in LAL mice (Compaan et al., 1993). Finally, SAL mice showed higher sensitivity to apomorphine-induced stereotyped behaviour (Benus et al., 1991b). Apomorphine acts on the dopaminergic system in the striatum, suggesting a difference between LAL and SAL in the sensitivity of this system. All together, these data indicate that LAL and SAL mice show a distinctly different organisation of the central nervous system, which might underlie the opposing behavioural coping styles displayed by the two mouse lines.

Table 2. Overview of the neuroendocrine and neurochemical differences between low-aggressive LAL and high-aggressive SAL males.

<table>
<thead>
<tr>
<th></th>
<th>LAL</th>
<th>SAL</th>
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<tbody>
<tr>
<td>Plasma corticosterone</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Hippocampal 5-HT&lt;sub&gt;1A&lt;/sub&gt; receptor</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Hippocampal mossy fibres</td>
<td>Large</td>
<td>Small</td>
</tr>
<tr>
<td>Septum AVP-ir fibre density</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Striatal dopamine sensitivity</td>
<td>Low</td>
<td>High</td>
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In view of the profound differences in behavioural traits as well as differences in corticosterone and 5-HT<sub>1A</sub> receptor, the LAL and SAL mice present an interesting animal model to study whether these differences are paralleled by differences in their stress system reactivity and their ability to cope with acute and chronic stressors.

4. Aim of the thesis

The relationship between alterations in the HPA and 5-HT systems and depressive behaviour is poorly understood, as well as how some individuals are more likely to develop depression than others under seemingly similar conditions. Understanding the individual differences in adaptive responses to stress may help to understand the relationship between stress and depressive illness. This thesis will study the ability to cope with different stressors in long (LAL) and short (SAL) attack latency mice at various levels of the HPA axis and 5-HT system. These two mouse lines are thought to represent the extremes of the two behavioural strategies (‘passive’ vs. ‘active’ coping style) which exist in normal populations of animals as well as man.
We hypothesize that this difference in behavioural coping style is associated with a differential response to stressors and, as a consequence, differential stressor susceptibility.

The aims of this thesis are:

i. To test the hypothesis that a difference in behavioural coping style is associated with a differential HPA and 5-HT functioning and hippocampal plasticity under basal and acute stress conditions

ii. To determine whether genetic selection for coping style is associated with a differential susceptibility to chronic stressors and whether this is accompanied by differences in HPA and 5-HT functioning and hippocampal plasticity

4.1 Experimental approach

LAL and SAL mice will be examined under baseline conditions and after acute stress in their response of markers of the HPA system and the 5-HT system. Exposure to a psychosocial stressor (sensory contact model) will be used as a model to investigate the ability of the mice to cope with a chronic stressor and to initiate changes which are also observed in human depression, namely: increased HPA activity, imbalance of hippocampal MR and GR and reduced expression of hippocampal 5-HT\textsubscript{1A} receptor. Finally, the effect of stress on hippocampal plasticity (by measuring hippocampal neurogenesis) was determined in LAL and SAL mice.

4.2 Outline of the thesis

In chapter 2, the two mouse lines are characterized for their neuroendocrine response pattern under basal and acute stress conditions to investigate whether a difference at a behavioural level is associated with a difference in HPA activity. Corticosterone and ACTH were measured at various time points to assess basal HPA activity during the light and dark phase. HPA reactivity was assessed by exposing the mice to an acute stressor (forced swimming for 5 min.). The mRNA expression of hypothalamic CRH and of hippocampal MR and GR was determined under basal and acute stress conditions.

In chapter 3, the capacity of LAL and SAL mice to cope with different chronic psychosocial stressors was studied. For this purpose, the sensory contact
model was used in which a male mouse (LAL or SAL) was continuously living opposite an aggressive (SAL) male for 25 days. One group of mice was additionally defeated by the SAL opponent for 21 consecutive days. Line- and stressor-specific differences on several physiological, neuroendocrine and behavioural parameters are described.

In chapter 4 it was hypothesized that the differential response of LAL and SAL mice to sensory contact stress was already present after a period of 5 days. Furthermore, the effect of type of opponent in the sensory contact model on this differential response was investigated. Therefore, a LAL or SAL male was continuously living opposite a SAL male, opposite a LAL male, or single. Line- and stressor-specific differences on several physiological, neuroendocrine and behavioural parameters are described.

Chapter 5 describes baseline and acute stress-induced differences in hippocampal cell proliferation rate in LAL and SAL mice, and the possible involvement of HPA hormones.

In chapter 6 differences between LAL and SAL mice are described at the level of 5HT₁₆ receptor expression and binding capacity and brain 5-HT turnover. The behavioural effect of two 5-HT₁₆ receptor agonists (8-OH-DPAT and S-15535) were study in LAL and SAL mice in the forced swim test. Finally, the effect of 8-OH-DPAT and S-15535 was determined on stress-induced 5-HT and 5-HIAA contents in several brain regions.
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