Dysregulation of the HPA-axis: implications for serotonin responses in the hippocampus

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Chapter 1

General Introduction
In this thesis, we investigated the effect of periods of prolonged high corticosteroid levels at different time-points in life on 5-HT neurotransmission in the hippocampus.

1.1. Hypothalamo-pituitary-adrenal axis and corticosteroids

1.1.1 Stress and the hypothalamic-pituitary-adrenal axis

Stress is a situation in which the body is ‘out of balance’, when homeostasis is threatened. When an organism is exposed to a stressor, several mechanisms are activated with the purpose to restore homeostasis. Stress activates processes in the central nervous system, particularly in the paraventricular nucleus of the hypothalamus (see Figure 1.1). When this brain region is stimulated by stress, it releases corticotrophin-releasing hormone (CRH) and its co-secretagogue vasopressin (VP). CRH and VP reach the pituitary gland, which is then stimulated to release adrenocorticotropic hormone (ACTH) into the circulation. Via the blood, ACTH reaches the adrenal glands, which in response to ACTH secretes cortisol (in humans) or corticosterone (in rodents) (Clarke and Davison, 1989). Corticosteroids exert numerous functions in the periphery and the central nervous system. In the periphery, corticosteroids are involved in energy mobilization (glycogenolysis), in the immune system, inhibition of bone and muscle growth, cell-growth and the cardiovascular system (McEwen and Stellar, 1993; Munck et al., 1984).

Corticosterone can pass the blood brain barrier and affect several brain regions that contain receptors for corticosterone. Via receptors in the hypothalamus and pituitary, corticosterone can exert an inhibitory effect on the production and release of CRH and ACTH respectively, the so-called negative feedback loop (Canny et al., 1989; Jacobson and Sapolsky, 1991; Plotsky et al., 1993; Sawchenko, 1987).

The activity of the hypothalamic-pituitary-adrenal axis (HPA-axis) is mainly determined by two factors, i.e. a combination of 1) rises due to stress with negative feedback efficacy superimposed on 2) normal circadian rhythm (Clarke and Davison, 1989). Both aspects can be largely altered in association with a number of diseases and disorders (see further: section 1.4).

Figure 1.1: Schematic representation of the HPA-axis. Upon stimulation of the HPA-axis, e.g. by stress, the paraventricular nucleus (PVN) secretes CRH and VP, which reach the pituitary. The pituitary releases ACTH into the bloodstream, which reaches the adrenal glands. In response to ACTH, the adrenal glands secrete corticosterone, which can enter the brain. Corticosteroids can exert their effect on brain regions containing corticosteroid receptors, like the hippocampus. A negative feedback loop exists via stimulation of corticosteroid receptors in the hypothalamus and pituitary.
1.1.2 Corticosteroids in the central nervous system

Next to the periphery, the central nervous system is also a target for corticosteroid action. Corticosteroids coordinate circadian events and restore disturbances in homeostasis induced by stress (McEwen et al., 1986; Munck et al., 1984). Corticosteroids are believed to play a role in long-term metabolic, affective and psychotic disease states (for review see Gold and Chrousos, 2002; McEwen and Stellar, 1993).

In the central nervous system, corticosteroids can exert their effect on all brain regions that contain receptors for corticosteroids. An area particularly rich in corticosteroid receptors is the hippocampus, which makes it a target for corticosteroids. In the hippocampus, corticosteroids affect neuronal survival, structure, gene expression and neuronal excitability and processes like learning and memory (Diaz-Brinton and Berger, 2000; Gould and Tanapat, 1999; Joëls, 1997; Kim and Diamond, 2002; McEwen, 2000; Sapolsky, 2000).

The hippocampus is a three-layered structure and can be subdivided in different areas: cornu ammonis 1-4 (CA1, CA2, CA3 and CA4), which contains (as principal cells) pyramidal neurons, and the dentate gyrus (DG), which contains granule cells. These areas are heavily interconnected. The dentate gyrus receives input from the nearby entorhinal cortex via afferents of the perforant path. In turn, there is a projection of mossy fibers from the DG that terminate in the CA3 area. The CA3 area sends projections via the Schaffer collaterals to the CA1 area. From the CA1 area and the subiculum, the loop is closed by returning projections to the entorhinal cortex (Diaz-Brinton and Berger, 2000; Lopes Da Silva et al., 1990; Sapolsky, 2000).

1.1.3 Two receptor systems for corticosteroids

Corticosteroids can bind to two receptor types, the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR). Both receptors exist in the periphery and the central nervous system, and have an identical structure in both periphery, like in the liver and kidney, and central nervous system (Arriza et al., 1987; Patel et al., 1989), although different receptor variants exist, e.g. of the GR (De Rijk et al., 2002). The MR is a high-affinity (Kd~0.5 nM) receptor which binds corticosterone at low concentrations. Therefore, the MR is almost always occupied for nearly 90% and has a function in maintaining homeostasis. The GR has an almost 10-fold lower affinity (Kd~5 nM) for corticosterone. Under basal conditions, i.e. during rest, the GR is occupied for about 10% (Reul and De Kloet, 1985). It becomes substantially occupied when the level of corticosterone rises, for example during the peak of the circadian rhythm (Reul and De Kloet, 1985) or during stress. Due to this differential occupation of the MR and GR, a difference in function has been proposed, with a more tonic inhibitory control function for the MR and a role for the GR in the negative feedback regulation of the HPA-axis during stress (De Kloet and Reul, 1987).

The MR and GR have a different distribution within the brain. The MR is highly expressed in limbic regions like the hippocampus, while the GR has a more widespread distribution in the brain, including hippocampus but also other brain regions like the PVN, suprachiasmatic nucleus, nucleus arcuatus-mediae eminence complex and the locus coeruleus. GR is also detected in the A2 nucleus and the raphe nuclei (Fuxe et al., 1985a; Fuxe et al., 1985b; Reul and De Kloet, 1985).

Both receptors are present in the hippocampus (Jacobson and Sapolsky, 1991). However, the areas of the hippocampus show a differential distribution of MR and GR. MR is present in high levels in all hippocampal subfields. The GR is highly expressed in
the CA1 and CA2 region, with low levels in CA3 and intermediate levels in the DG (Herman et al., 1989; Reul and De Kloet, 1985; Van Eeckelen et al., 1988). Co-localization of MR and GR is found in hippocampal pyramidal neurons (Van Steensel et al., 1996). This high expression of corticosteroid receptors in the hippocampus makes it possible for corticosteroids to modulate hippocampal and, indirectly, HPA-axis function over a wide range of corticosteroid levels (De Kloet et al., 1998).

1.1.4 Mechanism of action
Steroid receptors belong to the superfamily of nuclear receptors (Beato and Sanchez-Pacheco, 1996; Tsai and O'Malley, 1994). Corticosteroids can bind to intracellular receptors in the cytosol (Beato, 1989). These receptors are composed of a ligand-binding domain on the C-terminal, a DNA-binding domain with two zinc fingers motifs and an N-terminal domain involved in transrepression (Beato, 1989; Zilliacus et al., 1995). In particular, the protein complex of the GR has been extensively investigated. The receptor forms a multiprotein complex with heat shock proteins and an immunophilin (Jenkins et al., 2001; Kanelakis et al., 2002). The ligand binding domain of the receptor contains an association area for heat shock protein 90 and one of the dimerization domains.

Binding of corticosteroids to the receptors causes a rapid dissociation of the multiprotein complex and multiple phosphorylation steps are initiated (Beato, 1989; Tsai and O'Malley, 1994). This causes dimerization of activated receptor complexes and an increase in the affinity of the ligand-bound receptor for nuclear domains. Both homo- and heterodimers of MR and GR can be formed. Glucocorticoid responsive elements (GREs) are present on promoter regions of glucocorticoid-responsive genes (Zilliacus et al., 1995). Receptor dimers bind to the GREs of the nuclear DNA and via this route can initiate transcription (Stockner et al., 2003; Truss and Beato, 1993). Together with the DNA-binding domain, the N-terminal and C-terminal regions determine the transcriptional activity. Activated steroid receptors can not only exert their effects by binding to the DNA, but also indirectly by interaction with several transcription factors, like activating protein 1, nuclear factor-κB and cAMP-response element binding protein (Guardiola-Diaz et al., 1996; Pfahl, 1993; Ray and Prefontaine, 1994; Tronche et al., 1998). The binding of homo- or heterodimers to GREs leads to alterations in mRNA synthesis and subsequent protein synthesis.

1.1.5 Cellular actions in the CNS
In the hippocampus, corticosteroids generally do not affect passive membrane characteristics (Joëls and De Kloet, 1989; Kerr et al., 1989). Basal cell characteristics, like resting membrane potential and input resistance, show no steroid dependency, since these are comparable for control and adrenalectomized (ADX) animals (Beck et al., 1994; Joëls and De Kloet, 1989; Kerr et al., 1989). Yet, corticosteroids can affect several targets that play a role in neuronal excitability, for example ion channels, neurotransmitter systems and ion transporters (Joëls and De Kloet, 1995). Because the MR is occupied for nearly 90% during rest conditions, this receptor plays an important role in maintaining homeostasis of the cell. When predominantly the MR is occupied, there is no extensive Ca\(^{2+}\) influx, the accommodation and Ca\(^{2+}\)-dependent AHP are small, and neuronal excitability is increased (Beck et al., 1994; Joëls and De Kloet, 1990; Joëls and De Kloet, 1992). Upon depolarization, the amino-acid mediated transmission is stable and inhibitory input of serotonergic fibers is attenuated. All these factors contribute to the stability of the
network and the neuronal cells. Upon additional occupation of the GR, Ca\(^{2+}\) current, accommodation and AHP increase (Joëls, 2000; Karst et al., 1994; Kerr et al., 1992), and the inhibitory input from serotonergic innervation is no longer attenuated (Beck et al., 1994; Joëls and De Kloet, 1990; Joëls and De Kloet, 1992). While many of these properties will temporarily dampen neuronal excitability, in particular the increased Ca\(^{2+}\)-influx may in the end endanger the hippocampal cells and network integrity.

Interestingly, when no corticosteroids are present, i.e. after ADX, accommodation, Ca\(^{2+}\) current amplitude and AHP amplitude are also found to be large (Karst et al., 1994). This shows that the dose-dependency for at least several of the steroid-sensitive hippocampal cell properties forms a U-shaped relationship (Joëls and De Kloet, 1994). This U-shaped dependency is also seen for some neurotransmitter responses, like the serotonin response (Hesen et al., 1996).

### 1.2 Serotonergic system

#### 1.2.1 Function of serotonin in the CNS

In 1948, Rapport et al. discovered a potent vasotonic factor, serotonin (5-hydroxytryptamine, 5-HT) (Rapport et al., 1948). Serotonin turned out to be involved in several functions in the central nervous system. Serotonergic neurons can modulate electrical activity and afferent input responsivity in multiple targets by way of extensive collateralization. Via this extensive collateralization, serotonin has a modulatory role in autonomic, endocrine, motor and sensory functions and in complex emotional adaptive behaviour (Baumgarten and Grozdanovic, 2000). The widespread distribution of 5-HT fibers through the central nervous system accounts for the large variety of functions that can be modified by 5-HT, including motor output, body weight regulation, aggression, learning, sleep, circadian pattern, food intake and sexual activity. Serotonin is also involved in anxiety and affective disorders (Feldman et al., 1997; Smith and Cowen, 1997).

#### 1.2.2 Anatomy and projections of the 5-HT system

In the central nervous system, the dorsal raphe nucleus (DRN) contains the largest number of serotonergic cells. Together with the median raphe nucleus, the DRN contains about 80\% of the serotonergic neurons (Azmitia and Whitaker-Azmitia, 1991; Baumgarten and Grozdanovic, 2000). Serotonergic fibers have a very widespread distribution throughout the brain, innervating virtually all regions of the central nervous system, particularly cortex, limbic regions, basal ganglia and hypothalamus (Azmitia and Whitaker-Azmitia, 1991; Baumgarten and Grozdanovic, 2000).

The hippocampus receives a large projection of serotonergic neurons and is rich in serotonin receptors (Baumgarten and Grozdanovic, 2000; Segal and Landis, 1974; Zifa and Fillion, 1992). The projection of the raphe nuclei to the hippocampus shows a topographical organization. The DRN projects to the dorsal hippocampus, while the median raphe nucleus innervates the entire hippocampal system. Via the fimbria, projections from the raphe nuclei reach the stratum oriens and the stratum radiatum of the CA2-CA4 regions of the dorsal hippocampus (Baumgarten and Grozdanovic, 2000). The CA1 region of the hippocampus is reached by serotonergic fibers that run through the cingulate bundle (Lowry, 2002).
1.2.3 Serotonin receptor subtypes

At present, 14 different subtypes of serotonin receptors are known to be present in the brain. These receptors can be subdivided in different families, i.e. the 5-HT₁ (5-HT₁A, 5-HT₁B, 5-HT₁D, 5-HT₁E and 5-HT₁F), 5-HT₂ (5-HT₂A, 5-HT₂B and 5-HT₂C), 5-HT₃, 5-HT₄, 5-HT₅, 5-HT₆, and 5-HT₇ family (for review, see Barnes and Sharp, 1999; Hartig, 2000). All 5-HT receptors, with the exception of the 5-HT₃ receptor, belong to the superfamily of G-protein coupled receptors (Gerhardt and Van Heerikhuizen, 1997). The 5-HT₃ receptor is not coupled to a G-protein, but the receptor itself forms an ion channel across the membrane (Marić et al., 1991). Many of these 5-HT receptor subtypes are expressed in the hippocampus (Barnes and Sharp, 1999).

One of the best characterized 5-HT receptors is the 5-HT₁A receptor. It belongs to the 5-HT₁ family of receptors that is negatively coupled to adenylate cyclase via the Gi family of G proteins (Raymond et al., 1993). The 5-HT₁A receptor is linked to a G-protein coupled inwardly rectifying potassium (K⁺) channel (GIRK or Kir) (Andrade et al., 1986; Andrade and Nicoll, 1987; Sanders-Bush and Canton, 1995) and exerts different functions in the brain. In the DRN, the 5-HT₁A receptors act as somatodendritic autoreceptors, which on activation depress neuronal firing rate of the serotonergic neurons (Hamon, 2000). In other brain areas, like cortex and hippocampus, the 5-HT₁A receptor is located postsynaptically (Zgombick et al., 1989). Pyramidal neurons in the CA1 area show high levels of 5-HT₁A receptor mRNA and 5-HT₁A receptor binding (Aghajanian and Andrade, 2000). The 5-HT₁A receptor has been implicated in processes like development (Lauder, 1993; Miquel et al., 1994; Whitaker-Azmitia and Azmitia, 1994), sexual behaviour (Ahlenius et al., 1991) and temperature regulation (Bill et al., 1991). Furthermore, the 5-HT₁A receptor plays a role in anxiety and antidepressant treatment (De Vry, 1995; Hamon, 2000).

Other 5-HT receptors, like 5-HT₂ receptors, 5-HT₄ receptors and 5-HT₇ receptors, exert a depolarizing effect, which is slower in onset though more prolonged in time than actions via the 5-HT₁A receptors (Aghajanian and Andrade, 2000; Barnes and Sharp, 1999). Although these receptors may also form a target for corticosteroids (Birnstiel and Beck, 1995; Kuroda et al., 1994; Le Corre et al., 1997; Mendelson and McEwen, 1991; Watanabe et al., 1993; Yau et al., 2001), they were not directly examined in this thesis.

1.2.4 The 5-HT₁A receptor signalling pathway

Activation of postsynaptic 5-HT₁A receptors by 5-HT causes a conformational change in the G-protein and subsequently the potassium channel. The conformational change of the K⁺ channel causes opening of the channel, which results in an outflow of K⁺ ions and subsequently hyperpolarization of the cell (Andrade et al., 1986; Colino and Halliwell, 1987; Jahnsen, 1980; Sanders-Bush and Canton, 1995; Segal, 1980). In current clamp studies, activation of 5-HT₁A receptors therefore induces membrane hyperpolarization and decrease in resistance (see Box 1.1 and Figure 1.2).
Box 1.1: Intracellular recordings in the CA1 region of the hippocampus

The main technique used in this study is intracellular recording from CA1 pyramidal neurons in the hippocampus. The hippocampus is a very suitable substrate for this kind of study, because the structural organization of the hippocampus makes it possible to make transverse sections over the longitudinal axis. In these sections, the majority of the trisynaptic loop remains intact (Diaz-Brinton and Berger, 2000). CA1 pyramidal cells are impaled by a glass microelectrode. Via this electrode, basal cell characteristics like resting membrane potential, input resistance, inward rectification, membrane time constant, spike frequency accommodation and AHP can be recorded (as indicated by the arrows in typical example in Figure 1.2, left, above). The hippocampal slice is continuously perfused with artificial cerebrospinal fluid (ACSF). Addition of 5-HT (as indicated by the bar, left, above) to the ACSF makes it possible to record from the same cell before, during and after 5-HT application. 5-HT causes hyperpolarization of the cell and a decrease in input resistance due to activation of 5-HT$_{1A}$ receptors.

Figure 1.2: The hippocampus is dissected out of the brain and cut into 400 µM sections, in which intracellular recordings of CA1 pyramidal neurons are made by means of a glass microelectrode (Hall, 1992). The hippocampal slice is continuously perfused with artificial cerebrospinal fluid, to which 5-HT can be added. Perfusion of the slice with 10 µM 5-HT causes a hyperpolarization and a decrease in input resistance (inset left, above). Basal cell characteristics can be measured before, during and after 5-HT application, as indicated by the arrows. The insets below show examples for measurement of the inward rectification (left) and AHP (right).
The inwardly rectifying potassium channel, coupled to the 5-HT\textsubscript{1A} receptor, belongs to the Kir3 (or GIRK) family. This family consists of Kir3.1, Kir3.2, Kir3.3 and Kir3.4 subunits. The majority of the Kir3 channels are supposed to be heterotetramers containing the Kir3.1 subunit, although functional Kir3.2 homomers and Kir3.2/3.3 combinations have also been reported (Isomoto et al., 1997).

1.2.5 Potential modulators of 5-HT\textsubscript{1A} receptor signalling

Modulation of 5-HT\textsubscript{1A} receptor signalling can occur at different levels of the signal transduction pathway. For example, activity of the G-protein can be modulated by regulators of G-protein signalling (RGS). There are several types of RGS, RGS1-16. In cell cultures, co-expression of GIRK channels with RGS1, RGS3 or RGS4 accelerated GIRK current waveforms evoked by agonist activation of muscarinic m2 receptors or 5-HT\textsubscript{1A} receptors (Doupnik et al., 1997). RGS4 is of particular interest, because it is shown to be sensitive to corticosteroid treatment. Thus, in the PVN, mRNA expression for RGS4 is downregulated after acute and chronic exogeneous corticosterone administration. Also, chronic unpredictable stress decreases RGS4 mRNA expression (Ni et al., 1999).

Neuronal cell adhesion molecules (NCAM) may also play a modulatory role in 5-HT\textsubscript{1A} receptor signalling. NCAM deficient knock out mice show anxiolytic-like effects in response to lower doses of the 5-HT\textsubscript{1A} receptor agonists buspirone and 8-OH-DPAT. 5-HT synthesis, release and mRNA expression for the 5-HT\textsubscript{1A} receptors are not altered in NCAM--/-- mice, suggesting that NCAM might affect the GIRK channels that are coupled to the 5-HT\textsubscript{1A} receptor (Stork et al., 1999). Indeed, in cultured hippocampal neurons from NCAM-deficient mice, inwardly rectifying currents mediated by Kir3-channels are increased compared to wild type controls (Delling et al., 2002). Furthermore, NCAMs were shown to be sensitive to corticosteroid treatment and chronic stress, particularly in the prefrontal cortex, but also in the hippocampus (Sandi et al., 2001; Venero et al., 2002), suggesting that NCAMs might play a modulatory role in the effects of stress on 5-HT\textsubscript{1A} receptor signalling.

1.3 Interaction between corticosteroids and 5-HT

Interactions between the HPA-axis and the 5-HT system can occur at multiple levels. Both systems are interconnected and can influence each other. Serotonin can affect HPA functioning on different levels, e.g. hypothalamic, pituitary or adrenal level. For example, activation of 5-HT\textsubscript{1A} and 5-HT\textsubscript{2} receptors in the PVN of the hypothalamus increases the secretion of ACTH and corticosterone (Pan and Gilbert, 1992; Van de Kar et al., 2001). Thus, 5-HT increases the secretion of ACTH and corticosterone (Lesch et al., 1990; O'Keane and Dinan, 1991), an effect that is mimicked by application of 5-HT\textsubscript{1A} agonists (Lesch et al., 1990; Meltzer and Maes, 1994; Van de Kar, 2000). Also, application of 5-HT\textsubscript{2} agonists increases the secretion of ACTH (Cowen et al., 1990; Van de Kar et al., 2001). At the pituitary level, 5-HT-immunoreactive cells have been found (Carvajal et al., 1991; Szabat et al., 1998; Van de Kar, 2000). Moreover, 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) have been shown to exist in the adrenal cortex. 5-HT receptors in the adrenal cortex stimulate the secretion of corticosterone independently from the HPA-axis (Lefebvre et al., 1992). So, 5-HT is able to affect the HPA-axis. However, the 5-HT system itself is also affected by actions of the
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HPA-axis. These interactions are of special interest because clinical trials have shown that both physical and psychological stressors might precipitate the onset of depressive episodes (Post et al., 2000).

1.3.1 Acute effects of corticosteroids on the 5-HT system

Corticosteroids and stress can affect several aspects of the 5-HT system in the brain. First, stress is known to affect serotonin synthesis. Stress and corticosteroids are shown to increase the catalytic activity of tryptophan hydroxylase, the rate-limiting enzyme in 5-HT synthesis (Boadle-Biber et al., 1989; Singh et al., 1994; Singh et al., 1990). After ADX, this effect of stress is abolished (Singh et al., 1990). An increase in 5-HT synthesis in the DRN consequently increases the release of 5-HT in terminal areas (De Kloet et al., 1982; Van Loon et al., 1981). In accordance, an increase in extracellular 5-HT levels has been observed in the hippocampus after swim stress (Fujino et al., 2002; Linthorst et al., 2002). In the hippocampus, corticosteroids exert a stimulating influence on 5-HT release, which occurs through activation of the GR (Korte-Bouws et al., 1996).

Second, corticosteroids have an effect on the transcription of the 5-HT₁A receptor gene. In the situation that no corticosteroids are present, i.e. ADX, increases in 5-HT₁A receptor gene expression have been shown in all hippocampal subfields (Chalmers et al., 1994; Mendelson and McEwen, 1992; Zhong and Ciarranello, 1995), together with increases in 5-HT₁A receptor binding (Chalmers et al., 1993; Tejani-Butt and Labow, 1994). In the DG, occupation of the MR causes normalization in 5-HT₁A receptor gene expression, while additional occupation of the GR results in a decreased mRNA expression of the 5-HT₁A receptor (Meijer and De Kloet, 1994). The effect of high corticosteroid levels can be blocked partially by application of a MR antagonist, while a GR antagonist has no effect, indicating that the MR plays a crucial role in the effect of corticosteroids on 5-HT₁A receptor mRNA expression (Meijer and de Kloet, 1995). In the CA1 area of the hippocampus, high doses of corticosterone, sufficient to occupy the GR, or application of the GR agonist dexamethasone, attenuates the increase in 5-HT₁A receptor mRNA expression after ADX, but 5-HT₁A receptor mRNA levels remain higher when compared to SHAM (Chalmers et al., 1994). The 5-HT₁A gene is intronless and is shown, in a septal cell line, to contain a negative response element, that binds heterodimers with a higher affinity than MR or GR homodimers (Ou et al., 2001).

However, corticosteroids are also able to affect the expression of this gene via interaction with other genes and their respective protein products that can influence 5-HT₁A receptor function. For example, the GR can interact with two NF-κB elements in the promoter of the 5-HT₁A receptor gene, thereby repressing NF-κB-mediated induction of transcription (Wissink et al., 2000).

Third, corticosteroids affect functional responses to 5-HT. When the level of corticosteroids is low, for example during rest, when the MR is predominantly occupied, the response of CA1 pyramidal neurons to 5-HT is small (Beck et al., 1996; Hesen et al., 1996; Joëls and De Kloet, 1992; Joëls et al., 1991). When the level of corticosterone rises for example during stress, and the GR becomes additionally occupied, the 5-HT response increases (Joëls and De Kloet, 1992). The increase in 5-HT₁A receptor mediated responses after stress could be prevented by the application of the GR antagonist RU-38486 (Hesen et al., 1996). MR ligands suppress the 5-HT₁A receptor mediated hyperpolarization, while GR ligands don’t show this effect (Joëls et al., 1991). Interestingly, when no corticosteroids are present, during ADX, the response to 5-HT is also large (Hesen et al., 1996).

In mutant mice that cannot form GR homodimers, activation of the GR receptor does not induce an increase in 5-HT-induced hyperpolarization. The formation of GR homodimers is apparently necessary for the effects of high corticosteroid levels on the 5-HT response (Karst et al., 2000). The changes in functional response to 5-HT at different levels of circulating corticosteroids are probably not due to changes in the potassium channel that is coupled via a G-protein to the 5-HT$_{1A}$ receptor (Joëls et al., 1991). The 5-HT$_{1A}$ receptor shares the potassium channel with the GABA$_B$ receptor. Treatment with MR ligands does not change the response of GABA$_B$ receptors to baclofen, suggesting that the potassium channel is not affected by corticosteroids (Joëls et al., 1991). This is supported by the study of Muma and Beck (Muma and Beck, 1999), in which different treatments like ADX, aldosterone and high corticosteroid treatment, did not affect the expression of GIRK1 (Kir3.1) in the CA1 area.

### 1.3.2 Effects of prolonged elevations in corticosteroid level on the 5-HT system

The hippocampus is vulnerable to both chronic overexposure and chronic absence of corticosteroids (Arbel et al., 1994). Chronic absence of corticosteroids leads to apoptotic cell death particularly in the DG. After three days of absence of corticosteroids, DG neurons experience chromatin condensation, cell nuclear pyknosis, DNA fragmentation and cytoplasmic shrinkage (Sloviter et al., 1993; Sloviter et al., 1989). These effects can be prevented by the application of MR agonists (Sloviter et al., 1989; Woolley et al., 1991).

In humans, enduring levels of high corticosteroids are known to have a detrimental effect on hippocampal volume (Lupien et al., 1998). In rodents, a regression of apical dendrites and atrophy of CA3 neurons is seen (Magarinos and McEwen, 1995; Watanabe et al., 1992; Woolley et al., 1990). High corticosteroid levels increase hippocampal cell loss in primates and possibly in humans (Sapolsky et al., 1990). Although the effects are more pronounced in the DG and CA3 regions, also the CA1 is affected by high corticosteroid levels (Sousa et al., 2000). Factors involved in these damaging effects are thought to be an enhanced influx of Ca$^{2+}$ and alterations in the glutamatergic neurotransmission (Sapolsky, 2000).

The serotonergic system is also affected by prolonged elevation in corticosteroid level. Several studies show that 5-HT$_{1A}$ receptor mRNA expression and binding in the hippocampus are downregulated by chronic restraint (Watanabe et al., 1993) and chronic unpredictable stress (Lopez et al., 1998). ADX prevents this effect (Lopez et al., 1998), indicating the importance of corticosteroids in this effect. However, not all studies showed a downregulation of 5-HT$_{1A}$ receptor mRNA by chronic stress or chronic high levels of exogeneous corticosterone (Holmes et al., 1995; Karten et al., 1999; Lopez et al., 1998). Less data are available about the effects of chronic stress on functional 5-HT responses. At the start of this thesis, it was only known that 3 weeks of exogeneous corticosterone administration reduces 5-HT$_{1A}$ receptor-mediated hyperpolarization and attenuates the decrease in input resistance (Karten et al., 1999; Mueller and Beck, 2000).
1.4 Interaction between the HPA-axis and the 5-HT system in relation to major depressive disorder

1.4.1 Major depressive disorder

Disturbances of the serotonergic system are a key factor in depressive illness (Brown and Van Praag, 1991; Maes and Meltzer, 1995). This was first inferred from observations in the periphery, like low levels of plasma tryptophan, a reduced content of 5-HT and 5-HT transporters in blood platelets and low levels of the 5-HT metabolite 5-HIAA in the cerebrospinal fluid of depressed individuals (Meltzer, 1989). Also, studies in healthy subjects with or without a family history of depressive illness show that acute depletion of the 5-HT precursor tryptophan leads to lowering of mood (Benkelfat et al., 1994; Young et al., 1985). Genetic factors also play a role in the vulnerability of individuals to depression. A polymorphism in the 5-HT transporter is responsible for the outcome of treatment with antidepressants, like selective serotonin re-uptake inhibitors (SSRIs). In general, patients with the long (l) allele of the 5-HT transporter gene are more responsive to treatment with SSRIs, compared to patients with the short (s) allele (Eichhammer et al., 2003; Yu et al., 2002).

Deakin and Graeff (Deakin and Graeff, 1991) suggested that the 5-HT neurons of the DRN that project to the hippocampus have a function of maintaining adaptive behaviour in relation to aversive stimuli (Deakin and Graeff, 1991). Failure in this mechanism of defence could then result in the development of depression. Hippocampal atrophy and decreases in hippocampal size are features seen in depression, together with deficits in explicit memory. The degree of hippocampal size reduction correlates with the duration of depressive illness (Sheline et al., 1996).

Involvement of the HPA-axis in major depression is underscored by the observation that stressful life events often precede episodes of depression (Brown et al., 1994; McNaughton et al., 1992). In part of the depressive patients, abnormalities of the HPA-axis have been reported. In general, the HPA-axis is hyperactive in part of the depressive patients. HPA-axis hyperactivity is reflected in increased levels of ACTH, CRH and basal cortisol (Carroll et al., 1976; Young et al., 1991), hypertrophy of the adrenal glands (Amsterdam et al., 1987; Nemeroff et al., 1992; Rubin et al., 1996; Rubin et al., 1995) and enlargement of the pituitary (Axelson et al., 1992). These patients have impaired negative feedback, illustrated by dexamethasone nonsuppression of cortisol levels (Carroll et al., 1981; Holsboer, 1983; Kalin et al., 1982; Rush et al., 1996; Young et al., 1991). CRH-mediated stimulation of ACTH release is blunted (Holsboer et al., 1985; Ur et al., 1992). Some depressive patients show a flattening of the diurnal cortisol rhythm (Gartside et al., 2003).

1.4.2 Treatment in major depressive disorder

Several classes of antidepressant exist, i.e. tricyclic antidepressants, monoamine oxidase inhibitors and SSRIs. Long-term treatment with SSRIs increases the availability of releasable 5-HT, probably due to desensitization of 5-HT autoreceptors without alterations in responsiveness of 5-HT1A receptors in terminal areas (Blier et al., 1988). In agreement, chronic administration of SSRIs leads to a decrease in the functional activity of somatodendritic 5-HT1A receptors (Gartside et al., 2003). Tricyclic antidepressants are known to increase 5-HT1A receptor binding in the hippocampus, but not in DRN (Welner et al., 1989).
Antidepressants do not only affect the 5-HT system, but also cause alterations in the corticosteroid receptors in limbic areas. In rats, chronic treatment with antidepressants increases GR mRNA levels (Pepin et al., 1989), which was mirrored by increases in corticosteroid receptor binding sites (Reul et al., 1994). Desipramine restores MR/GR ratio in stressed animals, which may be a mechanism by which antidepressants enhance feedback and maintain low corticosterone levels, even in the presence of stress (Lopez et al., 1998).

Part of the depressive patients show abnormalities in HPA-axis functioning. For example, these patients and some of their family members show cortisol nonsuppression in the combined CRH/dexamethasone suppression test (Modell et al., 1998). Patients who continue to show cortisol nonsuppression after treatment of the disease have a higher risk for relapse (Greden et al., 1983). From these data, it became apparent that substances that act on the HPA-system can contribute to the clinical outcome of antidepressant treatment. Moreover, at the time this project started, it was already known that patients suffering of major depression, particularly those resistant to antidepressant treatment, show beneficial effects of supplemental treatment with steroid suppressing agents (Murphy et al., 1991; Wolkowitz et al., 1993).

1.5 Outline of this thesis

1.5.1 Aim of this thesis

Hyperactivity of the HPA-axis is a major risk factor for the precipitation of major depressive disorder in genetically predisposed individuals. Presently, it is unknown how HPA-axis hyperactivity, and the resulting hypercorticism, can result in the precipitation of clinical symptoms. We here hypothesize that corticosteroids reach this effect by a gradual attenuation of 5-HT responsiveness in limbic areas.

In this thesis, we investigated the effect of prolonged periods of high corticosteroid levels at different time-points in life on 5-HT neurotransmission in the hippocampus.

1.5.2 Questions

Much is known already about the interactions of the HPA-axis and the serotonergic system, particularly about the effects of acute corticosterone application or acute stress on 5-HT responsiveness in the hippocampus. Less data are available about the effects of long-term high levels of endogeneous corticosteroids on the functional response of 5-HT$_{1A}$ receptors in CA1 pyramidal neurons of the hippocampus. This knowledge is highly relevant in view of the possible clinical implications of long-term high corticosteroid levels in the precipitation of major depressive episodes. In this thesis, we investigate 5-HT$_{1A}$ receptor-mediated responses in different animal models, characterized by a prolonged period of high HPA-axis activity or by high HPA-axis activity during critical time-points in life.

In Chapter 2, animals are subjected to a 21-day protocol of unpredictable stress. After this time period, functional responses to 5-HT are recorded in pyramidal neurons of the CA1 area. Also, mRNA expression for the 5-HT$_{1A}$ receptor, the MR and the GR is studied.
In Chapter 3, we asked ourselves if stress early in life will affect the response to 5-HT later on, when rats have reached adulthood. For this purpose, the maternal deprivation protocol is used (Workel et al., 1997). On postnatal day 3, pups are separated from the dam and returned 24 hours later. After maternal deprivation, pups are left undisturbed until time of weaning at postnatal day 21. Rats are tested for their 5-HT responsiveness as young adults, at 3 months of age. 5-HT\textsubscript{1A} receptor mRNA expression is studied, as well as MR and GR mRNA expression in different hippocampal subfields.

In Chapter 4, we study the functional response to 5-HT in animals that are genetically selected on aggressive behaviour. These two mouse lines, the short attack latency (SAL) and long attack latency (LAL) mice, show differences in neuroendocrine parameters including the HPA-axis and in some parameters of the 5-HT system (Korte et al., 1996). Intracellular recordings are made to study the functional 5-HT response and again mRNA expression for the 5-HT\textsubscript{1A}-receptor, MR and GR is studied.

In Chapter 5, we try to elucidate some aspects of the mechanism, underlying attenuation of 5-HT responsiveness in animals exhibiting high levels of corticosteroids at some time in life. By means of in situ hybridization, a potential target is studied, i.e. NCAM.

The experimental findings are summarized and discussed in Chapter 6.

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**Box 1.2 Main questions of this thesis**

- Does chronic stress and hyperactivity of the HPA-axis affect 5-HT responses in CA1 pyramidal neurons of the hippocampus?
- Does this involve altered mRNA expression for the 5-HT\textsubscript{1A} receptor, MR and/or GR?
- If chronic stress attenuates 5-HT responsiveness, is this due to GR resistance?
- Do stressful early life events affect functional 5-HT responses in CA1 neurons later on in life?
- If so, does this involve decreased expression of 5-HT\textsubscript{1A} receptor mRNA, MR mRNA and/or GR mRNA in different hippocampal subfields?
- Is 5-HT\textsubscript{1A} receptor-mediated responsiveness changed between two mice lines that are genetically selected for aggression?
- Do mice genetically selected on aggression differ in mRNA expression profiles of the 5-HT\textsubscript{1A} receptor, MR and/or GR?
- Is the expression of NCAM\textsubscript{total} and NCAM\textsubscript{180} mRNA expression changed in the hippocampus of animals exposed to early life stress or long-term high levels of exogeneous corticosteroids when compared to control animals?

References are shown in the back of this thesis.