The stress system in the human brain in depression and neurodegeneration

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Abstract

Corticotropin-releasing hormone (CRH) plays a central role in the regulation of the hypothalamic-pituitary-adrenal (HPA)-axis, i.e., the final common pathway in the stress response. The action of CRH on ACTH release is strongly potentiated by vasopressin, that is co-produced in increasing amounts when the hypothalamic paraventricular neurons are chronically activated. Whereas vasopressin stimulates ACTH release in humans, oxytocin inhibits it. ACTH release results in the release of corticosteroids from the adrenal that, subsequently, through mineralocorticoid and glucocorticoid receptors, exert negative feedback on, among other things, the hippocampus, the pituitary and the hypothalamus. The most important glucocorticoid in humans is cortisol, present in higher levels in women than in men. During aging, the activation of the CRH neurons is modest compared to the extra activation observed in Alzheimer’s disease (AD) and the even stronger increase in major depression.

The HPA-axis is hyperactive in depression, due to genetic factors or due to aversive stimuli that may occur during early development or adult life. At least five interacting hypothalamic peptidergic systems are involved in the symptoms of major depression. Increased production of vasopressin in depression does not only occur in neurons that colocalize CRH, but also in neurons of the supraoptic nucleus (SON), which may lead to increased plasma levels of vasopressin, that have been related to an enhanced suicide risk. The increased activity of oxytocin neurons in the paraventricular nucleus (PVN) may be related to the eating disorders in depression. The suprachiasmatic nucleus (SCN), i.e., the biological clock of the brain, shows lower vasopressin production and a smaller circadian amplitude in depression, which may explain the sleeping problems in this disorder and may contribute to the strong CRH activation. The hypothalamo-pituitary thyroid (HPT)-axis is inhibited in depression. These hypothalamic peptidergic systems, i.e., the HPA-axis, the SCN, the SON and the HPT-axis, have many interactions with aminergic systems that are also implicated in depression. CRH neurons are strongly activated in depressed...
patients, and so is their HPA-axis, at all levels, but the individual variability is large. It is hypothesized that particularly a subgroup of CRH neurons that projects into the brain is activated in depression and induces the symptoms of this disorder. On the other hand, there is also a lot of evidence for a direct involvement of glucocorticoids in the etiology and symptoms of depression. Although there is a close association between cerebrospinal fluid (CSF) levels of CRH and alterations in the HPA-axis in depression, much of the CRH in CSF is likely to be derived from sources other than the PVN.

Furthermore, a close interaction between the HPA-axis and the hypothalamic-pituitary-gonadal (HPG)-axis exists. Organizing effects during fetal life as well as activating effects of sex hormones on the HPA-axis have been reported. Such mechanisms may be a basis for the higher prevalence of mood disorders in women as compared to men. In addition, the stress system is affected by changing levels of sex hormones, as found, e.g., in the premenstrual period, ante- and postpartum, during the transition phase to the menopause and during the use of oral contraceptives. In depressed women, plasma levels of estrogen are usually lower and plasma levels of androgens are increased, while testosterone levels are decreased in depressed men. This is explained by the fact that both in depressed males and females the HPA-axis is increased in activity, parallel to a diminished HPG-axis, while the major source of androgens in women is the adrenal, whereas in men it is the testes. It is speculated, however, that in the etiology of depression the relative levels of sex hormones play a more important role than their absolute levels. Sex hormone replacement therapy indeed seems to improve mood in elderly people and AD patients.

Studies of rats have shown that high levels of cumulative corticosteroid exposure and rather extreme chronic stress induce neuronal damage that selectively affects hippocampal structure. Studies performed under less extreme circumstances have so far provided conflicting data. The corticosteroid neurotoxicity hypothesis that evolved as a result of these initial observations is, however, not supported by clinical and experimental observations. In a few recent postmortem studies in patients treated with corticosteroids and patients who had been seriously and chronically depressed no indications for AD neuropathology, massive cell loss, or loss of plasticity could be found, while the incidence of apoptosis was extremely rare and only seen outside regions expected to be at risk for steroid overexposure. In addition, various recent experimental studies using good stereological methods failed to find massive cell loss in the hippocampus following exposure to stress or steroids, but rather showed adaptive and reversible changes in structural parameters after stress.

Thus, the HPA-axis in AD is only moderately activated, possibly due to the initial (primary) hippocampal degeneration in this condition. There are no convincing arguments to presume a causal, primary role for cortisol in the pathogenesis of AD. Although cortisol and CRH may well be causally involved in the signs and symptoms of depression, there is so far no evidence for any major irreversible damage in the human hippocampus in this disorder.

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1. Introduction

1.1. Corticotropin-releasing hormone (CRH) neurons in the PVN

Corticotropin-releasing hormone (CRH) is a crucial neuropeptide in the regulation of the hypothalamic-pituitary-adrenal (HPA)-axis, i.e., the final common pathway in the stress response. In addition, it has various central effects, including cardiovascular
regulation, respiration, appetite control, stress-related behavior and mood, cerebral blood flow regulation (Lehnert et al., 1998) and stress-induced analgesia (for review see Swaab, 2003). The hormonal end-product of the HPA-axis, cortisol, is one of the most powerful endogenous feedback compounds on the pro-inflammatory signal transduction machinery (Rivest, 2001). The chronic stress response further involves a prominent activation of the HPA-axis and autonomic nervous system, alterations in levels of anxiety, a loss of cognitive and affective flexibility, and an inhibition of vegetative processes that are likely to impede survival of the organism during a life-threatening situation, like sleep, sexual activity and endocrine programs for growth and reproduction (Gold and Chrousos, 2002; Swaab, 2003).

Corticosteroids act on many organs and various brain areas through two types of receptor, i.e., mineralocorticoid receptors (MR) and glucocorticoid receptors (GR), which have specific and selective distributions in the brain (Reul and De Kloet, 1985). GRs have been found in multiple brain regions relevant to cognition, i.e., the hippocampus, amygdala and prefrontal cortex. The hippocampus is especially important for declarative and spatial memory, the amygdala is critical for emotional memory and the prefrontal cortex is involved in working memory. These regions are not only target regions of glucocorticoid (GC) action but are also actively involved in feedback regulation of the HPA-axis; GCs exert negative feedback at the same levels at which their release was earlier initiated, i.e., the pituitary, hypothalamus (leading to reduced HPA activity) and the hippocampus. In addition, other areas, such as the cortex, contain high densities of GR, and positive feedback can be exerted, at the level of the amygdala, the prefrontal cortex and the brain stem (locus coeruleus), interfering with HPA activity or reactivity and stress effects on memory (e.g., Quirarte et al., 1997; Roozendaal, 2002; Fuchs et al., 2004).

When released together into the portal capillaries, the ACTH-releasing activity of CRH is strongly potentiated by vasopressin (Gillies et al., 1982; Rivier and Vale, 1983). It would be interesting to know whether a similar potentiating effect exists for its central effects. In the pituitary, vasopressin triggers ACTH release through a specific receptor subtype termed V3 or V1b, which is almost exclusively expressed by pituitary corticotrophs and some corticotroph tumors (René et al., 2000). Oxytocin inhibits ACTH-release, which has been confirmed in humans (Legros, 2001), providing yet another example of opposite actions of vasopressin and oxytocin. In women, both suckling and breast stimulation induces a significant increase in plasma oxytocin levels and a decrease in plasma ACTH levels, which agrees with the inhibitory influence of oxytocin on ACTH and cortisol secretion in humans (Chiodera et al., 1991). CRH and vasopressin mediate ACTH release via different second messenger systems. CRH activates G protein-linked adenylate cyclase, leading to cAMP formation and protein-kinase-A activation. Vasopressin, however, activates only phospholipase C. Prostaglandins may have specific interactions with both pathways. In this respect it is interesting that aspirin, an inhibitor of prostaglandin synthesis, significantly reduces the cortisol response to vasopressin in humans (Nye et al., 1997).

Following different types of corticosteroid treatment in different disorders or during the presence of high levels of endogenous corticosteroids produced by a tumor, we found, in postmortem tissue, not only that CRH-expressing neurons are hard to detect, but also that vasopressin expression in the SON and PVN is strongly decreased. Oxytocin neurons were not affected (Erkut et al., 1998, 2004) further illustrating that in the human brain, selective negative cortisol feedback is present in the CRH cells and in cells that co-express
vasopressin. Secondly, it stresses the importance of information on medications that affect the HPA-axis for studies of postmortem brain tissues.

CRH immunoreactivity is present in the parvicellular neurons of the PVN of the human hypothalamus. Also the CRH-vasopressin double-immunopositive neurons are parvicellular (Raadsheer et al., 1993; Raadsheer, 1994; Fig. 1). CRH fibers innervate the median eminence, where CRH is released into the portal vessels, while other CRH fibers run into the brain (Raadsheer et al., 1993) and are found to innervate LHbH neurons in the infundibular nucleus, that could form a possible substrate for CRH control of reproductive functions (Dudás and Merchenthaler, 2002b). CRH-positive cells and fibers are present in the human brain beginning at fetal weeks 12–16 (Bresson et al., 1987). Originally, only few CRH-expressing neurons were found in the rostral PVN (Pelletier et al., 1983; Raadsheer et al., 1993). Later, a more sensitive technique revealed CRH neurons, not only in the most rostral part of the PVN, but also in the medially situated parvicellular nucleus and posterior subnucleus of the PVN (Koutcherov et al., 2000), as well as in other brain areas such as the thalamus (Bao et al., 2005, in press).

1.2. Effects of gender, aging, and disease

Gender profoundly affects the outcome of the dexamethasone-CRH test: females, regardless of age, have an increased hormonal secretion in comparison to males (Heuser
et al., 1994a). Cortisol levels are 19% higher in women than in men (Laughlin and Barrett-Connor, 2000), while the circadian timing of cortisol secretion changes with the stage of the menstrual cycle (Parry et al., 2000). In postmortem CSF we found higher cortisol levels in women than in men, both in Alzheimer’s disease patients and in controls (Swaab et al., 1994; Erkut et al., 2004). In women the CRH neurons are presumed to be more active than in men (Antonijevic et al., 1999), while the cortisol production rate is clearly higher in men than in women (Vierhapper et al., 1998; Shamim et al., 2000). Sex hormone effects on the HPA-axis and on cortisol binding proteins may at least partly explain these differences. Although the sexual dimorphism in cortisol metabolism does not depend on estrogens (Toogood et al., 2000), ovarian steroids do increase HPA-axis activity, enhance the HPA-axis response to psychological stress, and sensitize the hypothalamic-pituitary-ovarian-axis to stress-induced inhibition (Kirschbaum et al., 1996; Roy et al., 1999). In premenopausal women a significant reduction of ACTH and cortisol is found after ovariectomy, while the response of ACTH, but not of cortisol, to CRH was reduced (De Leo et al., 1998).

The exact way in which the activity of CRH neurons is modulated by sex hormones is currently under investigation. In addition, sex differences in free cortisol, may, at least partly, be explained by estradiol-induced changes in cortisol-binding protein levels (Kirschbaum et al., 1999). During aging, clear signs of activation of CRH neurons were observed in both sexes. Total numbers of CRH-producing neurons and the proportion of vasopressin co-expressing CRH neurons went up strongly from the age of 40 onwards (Raadsheer et al., 1994a,b; Figs. 2 and 3; see below). Although the differences are

![Fig. 2. Linear regression between age and corticotrophin CRH cell number in the PVN estimated by the dissector method. Filled circles and solid lines indicate control subjects; open circles and dashed lines indicate AD patients. A significant correlation was found between age and absolute CRH cell number for control subjects (rho = 0.66, p = 0.02). In AD patients, the age effect was almost significant (rho = 0.53, p = 0.06) (from Raadsheer et al., 1994a, Fig. 3, with permission).](image-url)
relatively small, cortisol CSF and plasma levels do increase progressively between 20 and 80 years of age (Van Cauter et al., 1996; Guazzo et al., 1996; Swaab et al., 1996; Laughlin and Barrett-Connor, 2000). Furthermore, sex-dependent effects of aging on HPA-axis hormone levels have been described. In women, higher mean hypothalamic CRH levels were found than in men (Frederiksen et al., 1991), while also the adrenal response to CRH is elevated in elderly women (Greenspan et al., 1993). Moreover, in response to a cognitive challenge, older women exhibited greater increases of salivary free cortisol than older men, whereas in young adults it was the men who exhibited greater increases (Seeman et al., 2001). On the basis of the total number of cells expressing CRH, the total number of CRH neurons showing vasopressin colocalization and the increased amount of CRH-mRNA in the PVN, depressed patients showed a much stronger CRH neuron activation than AD patients. These changes in the CRH neurons may be responsible for at least part of the signs and symptoms of depression (Raadsheer et al., 1994c, 1995; see below).

1.3. Depression

1.3.1. Neurobiology of depression

Major depressive disorders are considered to have a neurochemical basis in multiple signaling pathways in different brain areas, and indeed various regionally selective impairments of structural plasticity have been reported (Manji and Duman, 2001). At least five interacting hypothalamic peptidergic systems are currently considered to be involved in symptoms of depression, as well as three aminergic transmitter systems that innervate the hypothalamus.
1.3.2. Depression and neuropeptides

Depressive illness is presumed to result from an interaction between the effects of environmental stress and genetic/developmental predisposition. The HPA-axis, a key system in control of the stress response, is considered to be the ‘final common pathway’ for a major part of the depressive symptomatology, while also subsequent changes in the serotonin system are involved. Although the set point of HPA-axis activity and of other central systems is programmed by genotype, it can be changed to another level by developmental influences and early negative life events. Long-lasting hyper(re)activity of the CRH neurons, resulting in increased stress responsiveness and reflecting a glucocorticoid resistant state, is commonly seen in depressed individuals (De Kloet et al., 1997; Heim and Nemeroff, 2001; Pariante, 2003). Prenatal environmental stressors of a chemical nature, such as nicotine exposure due to smoking of the pregnant mother, may sensitize a subject for developing depression in later life, especially children who were either light or heavy at birth (Clark et al., 1996; Clark, 1998). Observations in humans further indicate that aversive experiences, both in utero and in the neonatal period, result in sustained HPA-axis activation and in sensitization of emotional and HPA-axis responses to subsequent stress. Maternal stress beginning at infancy and subsequent stress during childhood is accompanied by a sensitization of the child’s HPA-axis response to subsequent stress exposure (Holboer and Barden, 1996; Checkley, 1996; Nemeroff, 1996; Clark, 1998; Kraemer, 1997; Carlson and Earls, 1997; Meaney, 2001; Essex et al., 2002). Stressful life events such as bereavement, child abuse, and early maternal separation are also risk factors for depression, anxiety disorder, or both. Childhood physical or sexual abuse are important early stressors that may predispose individuals to adult onset depression accompanied by a permanent hyperactivity of the HPA system.

In addition, small size at birth is associated with an alteration in the set-point of the HPA-axis and an increased cortisol responsiveness and risk of depression in adulthood (Phillips, 2001; Thompson et al., 2001). Interestingly, the risk of depression also remains elevated, notably for decades, following head injury in adulthood. This risk seems to be the highest in those who suffered a more severe head injury (Holsinger et al., 2002).

Almost all environmental and genetic risk factors for depression appear to correlate with increased HPA-axis activity in adulthood. On the other hand, when patients or animals are treated with antidepressants, electroconvulsive therapy, or when they show spontaneous remission, the HPA-axis function returns to normal (Nemeroff, 1996).

In adulthood, the HPA-axis is not only activated by stressful events, but also by pro-inflammatory cytokines such as interleukin-6 or exogenous interferon gamma that activates such cytokines (Cassidy and O’Keane, 2000). Indeed, the CRH neurons of the PVN that regulate the HPA-axis are strongly activated in depression, while in the majority of the depressed patients, dexamethasone resistance is prominent (see below).

In addition to these clinical observations, changes in the brain centres that initiate and control the stress response, such as the hypothalamus, have also been reported. In the PVN of patients with major depression or bipolar disorder, vasopressin and oxytocin neurons are activated as well, which may have functional consequences for HPA-axis reactivity, since vasopressin potentiates the effects of CRH. Von Bardeleben and Holboer (1989) already postulated that increased release of vasopressin into the portal capillaries in depression would enhance the action of CRH at the pituitary level. Consistent with this hypothesis,
CSF CRH and vasopressin levels are indeed associated with a diminished response of the pituitary to CRH (Newport et al., 2003). Moreover, depression is associated with an enhanced pituitary vasopressinergic responsivity (Dinan et al., 1999). Because of their central effects, the parallel activation of oxytocin neurons in depression has been connected to eating disorders in depression (Purba et al., 1996). Interestingly, the supranaoptic nucleus also shows enhanced vasopressin mRNA production in depression (Meynen et al., in preparation) that may be related to the increased plasma levels of vasopressin (Van Londen et al., 1997, 1998b, 2001) and to an enhanced suicide risk (Inder et al., 1997).

The possibility that chronically elevated vasopressin levels are involved in the induction of depressive symptomatology is further supported by the case of a 47-year-old man with an esthesioneuroblastoma with paraneoplastic secretion of vasopressin, that was associated with the onset of a first episode of major depression. The man displayed chronically elevated plasma vasopressin levels due to vasopressin secretion by the tumor. Depressive symptoms improved markedly after surgical resection of the tumor and subsequent normalization of plasma vasopressin levels. However, surprisingly, the chronically elevated vasopressin levels also led to a marked desensitization of the HPA-axis, suggesting that changes in feedback had occurred as well (Müller et al., 2000b).

The SCN, the clock of the hypothalamus, normally shows strong circadian and circannual variations in neuronal activity (Hofman and Swaab, 1992b, 1993a) which are supposed to be related to circadian and circannual fluctuations in mood and to sleeping disturbances in depression. In addition, biological rhythms are disturbed in depression (Van Londen et al., 2001). A disorder of SCN function, characterised by an increased amount of vasopressin, the decreased amount of vasopressin mRNA in this nucleus (Fig. 4), and diminished circadian fluctuation of vasopressin mRNA may not only be the basis of the circadian and sleeping disorders in depression, but may also contribute to hyperactivity of the CRH neurons, since this nucleus extends direct projections to the PVN (Dai et al., 1998; Zhou et al., 2001; Kalsbeek et al., 1992). Decreased activity of the SCN in depression is presumed to be due to the increased circulating plasma cortisol levels that are known to inhibit SCN function (Liu et al., unpublished data). Desan et al. (2000) reported a single nucleotide polymorphism in the CLOCK gene, that goes together with a preference for activity in the evening, but was not associated with depression. A polymorphism in the clock gene NPAS2 appeared, however, to be associated with seasonal affective disorder (Johansson et al., 2003).

Furthermore, depressed patients have alterations in their HPT-axis (Musselman and Nemeroff, 1996), as both basal TSH and thyroxin levels were found to be altered in melancholic and major depressed patients (Maes et al., 1993b).

1.4. Amines in the hypothalamus and depression

Patients with depression have alterations in serotonin (5HT), noradrenaline and dopamine production by the brain (Lambert et al., 2000). The hypothalamus is strongly innervated by the brain systems producing these transmitters, that are considered to play important roles in the pathogenesis of depression. Indeed, increased dopamine levels were observed in the hypothalamus of suicide victims who died as a result of carbon monoxide poisoning or drug overdose. However, the possibility that these changes are secondary to
hypoxia or due to drug effects should be considered (Arranz et al., 1997). Impulsive aggression and suicidal behavior have been related to a decreased serotonergic activity (Coccaro, 1992), but whether the serotonergic innervation of the hypothalamus is crucial in this respect, awaits further investigation. Some conditions, such as major depression, violent suicide and seasonal affective disorder are presumed to be related to the rhythmicity of serotonin function (Cappiello et al., 1996). With respect to the seasonal and circadian fluctuations in mood, the circannual and day/night fluctuations in hypothalamic content of 5HT and dopamine (Carlsson et al., 1980a) may be of particular interest. The rhythmic circadian and circannual fluctuations in amines suggest that the hypothalamic SCN may drive the aminergic neurotransmitter system rather than the other way around. Noradrenaline is found to be increased in the hypothalamus of depressed patients that committed suicide and in alcoholic patients that committed suicide (Moses and Robins, 1975). Depression in suicide victims was also found to be related to the presence of supersensitive beta 2A-adrenoceptors in the hypothalamus and prefrontal cortex (Meana et al., 1992; Oren et al., 1996), although other data indicated a decrease in postsynaptic α2A-adrenergereceptor responsiveness in depression (Mokrani et al., 1997).

With respect to genetic factors, polymorphisms in the serotonin transporter promotor and in genes encoding the serotonin receptors 5-HT2A and 5-HT2C and tryptophan hydroxylase do not appear to play a major role in the pathogenesis of seasonal affective disorder (Johansson et al., 2001; see below), although others consider them vulnerability factors (Praschak-Rieder et al., 2002). Carriers of the 5-HT2C ser allele were 12 times more likely to have major depression in Alzheimer’s disease (Holmes et al., 2003). A
serotonin transporter promoter polymorphism (Sher et al., 1999) and α-7 nicotine receptor polymorphisms (Stassen et al., 2000) were said to be associated with this type of depression. In addition, mutations or allelic variations in clock genes, such as CLOCK, have been proposed to contribute to the symptoms of depression in seasonal affective disorder and subgroups of major depression (Bunney and Bunney, 2000). Other genetic variations that are possibly implicated in the vulnerability to develop depression, such as polymorphisms or mutations in the glucocorticoid receptor (Holsboer et al., 1995; Holsboer, 2000; Pariante and Miller, 2000), will be discussed in more detail below. In addition, many other interactions are possible between the peptidergic and aminergic networks and endocrine changes in depression.

1.5. Other factors involved in depression

Studies of children of prisoners subjected to starvation in the Dutch winter of 1944–1945, prenatal famine in middle or late gestation is a risk factor for major depression. These effects were demonstrated for men and women and for unipolar and bipolar affective disorders (Brown et al., 2000). Whether dysfunction of the HPA-axis exists in these subjects has not been investigated.

Several hormonal factors may also be involved in depression. For instance, prenatal DES exposure increases the risk for depression (Meyer-Bahlburg and Ehrhardt, 1987), while growth hormone deficient children are at risk for depression and react favourably to growth hormone treatment (Stabler et al., 1996). Hypertension is frequently accompanied by feelings of hopelessness (Everson et al., 2000), that may relate to the increased activity of the CRH neurons found in this condition (Goncharuk et al., 2002). Depression is also commonly observed after stroke. The severity of the mood disorder in this condition is increased particularly in patients with left prefrontal frontal cortex lesions or with right posterior-dorsal lesions (Robinson et al., 1984; Iacoboni et al., 1995).

Postmortem studies have further provided morphological evidence for the involvement of the prefrontal cortex in depression; cell loss takes place in the prefrontal cortex, while cell atrophy occurs in the dorsolateral and orbitofrontal cortex (Rajkowska, 2000). Moreover, numbers of GFAP positive astrocytes are decreased in the dorsolateral prefrontal cortex of young depressed patients but increased in older depressed patients (Miguel-Hidalgo et al., 2000). PET and SPECT studies have further shown that bilateral hypometabolism occurs in the orbital-inferior prefrontal lobe of most types of depression, regardless of the origin of the disorder (George et al., 1993; Mayberg, 1994; Morris et al., 1996; Galynker et al., 1998). Whether these metabolic changes in the cortex of depressed patients are cause or effect of the disorder remains a matter of debate. Increased glucocorticoid levels are known to inhibit prefrontal cortex metabolism (Brunetti et al., 1998; Fulham et al., 1995). In addition, glucocorticoid receptor dysregulation is found in the neocortex and hippocampus of patients with depression (Webster et al., 2002; Miller and Pariante, 2001). This is consistent with the normalization of glucocorticoid receptor expression levels found after antidepressant treatments in animal studies (Yau et al., 1995, 2002; Johansson et al., 1998). Furthermore, the prefrontal cortex inhibits the HPA-axis, as is clear from lesion studies, particularly of the left prefrontal cortex; a lesion in that area appears to go together with symptoms of depression and hypercortisolism. On the other
hand, the hypercortisolism that occurs in depression will inhibit prefrontal cortex activity. Together, these two effects might even reinforce each other (Swaab et al., 2000). In depression pathogenesis, the interaction between the prefrontal cortex and the HPA-axis is therefore of crucial importance.

1.6. HPA-axis and depression

Patients with major depression and patients with bipolar disorder show a much stronger CRH neuron activation than aged controls or Alzheimer’s disease patients do, as appears from the four-fold increase in total number of CRH expressing cells, the increased total number of CRH neurons that show vasopressin colocalization, and the increased amount of CRH-mRNA in the PVN (Raadsheer et al., 1994c, 1995; Figs. 5 and 6).

In addition to these robust and well-established indications for a hypothalamic hyperdrive, there are various other measures that point to a causal and primary role for an increased HPA-axis activity in depression (Pariante, 2003). First, it is generally known that stressful life events are among the most potent factors that can trigger depressive episodes

Fig. 5. Total hybridization signal for human CRH-mRNA (arbitrary units) in the PVN. Bars indicate median values per patient group. The PVN of the AD \( (n = 10) \) contained significantly more (MW: \( U = 23.0, W = 0.78, Z = -2.0, p = 0.04 \)) CRH-mRNA than that of comparison subjects \( (n = 10) \). The amount of radioactivity in depressed patients \( (n = 7) \) was significantly higher than in comparison cases (MW: \( U = 7.0, W = 91.0, Z = -2.7, p = 0.006 \)) and AD patients (MW: \( U = 23.0, W = 0.78, Z = -2.0, p = 0.05 \)) (from Raadsheer et al., 1995, Fig. 2, with permission).
Second, elevated plasma and salivary cortisol and cortisone levels, increased urinary free cortisol excretion, disturbed dexamethasone suppression, decreased corticosteroid receptor function, an enhanced adrenal response to ACTH, a blunted pituitary ACTH response to CRH as well as adrenal and pituitary enlargement are commonly observed in patients suffering from depression (Scott and Dinan, 1998; Krishnan et al., 1991; O’Brien et al., 1996; Rubin et al., 1996; Modell et al., 1997; Maes et al., 1998; Weber et al., 2000; Holsboer, 2000). The combined dexamethasone/CRH test does not only identify, with high sensitivity, a dysfunction of the HPA-axis in depression; the elevated cortisol response in the test also correlates with a 4–6-fold higher risk for relapse than in individuals who had a depression but subsequently showed a normal cortisol response (Zobel et al., 2001). It is of particular interest that the adrenal weight increase in suicide victims was accounted for specifically by increases in the weight of the left adrenal (Szigethy et al., 1994). A unilateral activation is consistent with the proposed functional importance of adrenal innervation for regulation of the sensitivity for ACTH of these glands in rat (Buijs et al., 1999).

These abnormalities are, in part, normalized after (pharmacotherapy (Fig. 7); normalization of the axis in fact decreases relapse probability. Moreover, studies in high-risk probands of patients with major depression have shown that abnormalities in HPA-axis function already exist prior to the onset of the clinical symptoms, suggesting that such abnormalities not only correlate but can in fact precipitate depressive episodes (Holsboer, 2000). If so, one would predict that treatment aimed directly at interfering with the consequences of HPA-axis abnormalities, would reverse the clinical symptoms (Fig. 7).
Recent studies in patients with psychotic depression indeed support this concept, as treatment with high doses of GR-antagonist ameliorated most clinical symptoms (Belanoff et al., 2002). The smaller pituitary volumes found in one study in patients with bipolar disorder (Sassi et al., 2001) and the urinary hyposecretion of cortisol in a small
group of elderly depressed patients (Oldehinkel et al., 2001) remain, however, difficult to explain.

It should be noted that there is considerable individual variability in the degree of HPA- axis activation (see also Raadsheer et al., 1995; Fig. 5). The increased basal plasma cortisol levels are present in only some 25% of the subjects with major depression, while 66% show non-suppression of cortisol to dexamethasone (Young et al., 2001). In a recent study, Brunner et al. (2002) did not find any indication for an activation of the HPA-axis in depressed patients, including those that attempted suicide, on the basis of the dexamethasone/CRH test, or of plasma cortisol levels. Indeed, most patients with major depression are not hypercortisolemic when studied cross-sectionally; however, this does not exclude clinically significant episodes of excessive exposure to glucocorticoids. In addition, part of such differences may be attributed to methodological differences as to whether or not patients had been admitted to psychiatric wards or were living at home when tested. Also, urinary free cortisol excretion may be elevated for 21 days per month in depressed patients, compared to 4–5 days per month in control subjects. In addition, plasma cortisol levels strongly fluctuate during the circadian cycle, and elevated or normal levels can thus be measured depending on the time of day in depressed patients (Gold et al., 2002). Moreover, the HPA-axis is not overtly abnormal in chronic depression, not even when tested with a sensitive dexamethasone/CRH test (Watson et al., 2002). Subjects with psychotic major depression have higher cortisol levels throughout the afternoon than subjects with non-psychotic major depression, which may also contribute to the variability in cortisol levels (Belanoff et al., 2002). In younger patients suffering from depression, adrenal steroid abnormalities are also apparent when dehydroepiandrosterone (DHEA) is determined, a developmentally sensitive steroid that is a precursor for testosterone and estrogens (Goodyer et al., 1996, 1998). Scott et al. (1999a) found lower plasma levels of DHEA-S but not of DHEA in depressive patients. Elevated baseline cortisol levels are not only related to mood disorders, but also to cognitive impairment in depressed patients (Van Londen et al., 1998a).

In some studies CRH levels in CSF are higher in major depression than in mania, anxiety or controls (Banki et al., 1992; Mitchell, 1998; Wong et al., 2000). The increased CSF-CRH levels may, however, also be due to the stress of the anticipation of the lumbar puncture or the puncture itself (Geraciotti et al., 1992). When lumbar CSF was continuously sampled, thereby avoiding a stress response, CSF-CRH levels were found to be strikingly reduced in depressed patients (Geraciotti et al., 1992). In postmortem cisternal CSF, elevated CRH levels were measured in suicide victims that had an underlying depression (Arató et al., 1989). However, Brunner et al. (2002) did not find a difference in lumbar CSF CRH in drug-free, depressed suicide attemptees compared to non-attemptees in this study, and could thus not confirm the earlier observation of increased CSF CRH in suicide victims by Arató et al. (1989). Although there is a close association between CSF-CRH levels and alterations in the HPA-axis in depression (Newport et al., 2003), one may wonder what proportion of the CSF-CRH is actually derived from the PVN. CRH is not only produced by the PVN, but also in extrahypothalamic areas and in the spinal cord, where differential changes may occur that will affect the mean CSF values. As such, CSF levels of CRH are unlikely to represent PVN activation directly. At least CRH levels in extra-hypothalamic sites may return to near normal during remission (Mitchell,
indicating their role as a state marker. CSF-CRH may thus, at least for the largest part, represent fluctuations in extra-hypothalamic CRH derived from the neocortex, limbic and brainstem regions rather than in hypothalamic CRH (Mitchell, 1998; Arborelius et al., 1999; Gottfries et al., 1995; Vythilingam et al., 2000; Galard et al., 2002).

The locus coeruleus of depressed subjects contains elevated CRH concentrations. This brain area receives CRH input from the central nucleus of the amygdala and from pontine-medullary projections (Bissette et al., 2003). CSF-CRH levels are increased in anxiety and depression, even though there is super-suppression of cortisol following dexamethasone in anxiety and non-suppression in depression (Boyer, 2000). These observations further indicate that CSF-CRH levels do not necessarily reflect HPA-axis activity. Another observation supporting the idea that CRH in CSF is derived from other sources than the HPA-axis is that, in spite of the fact that in post-traumatic stress disorder the HPA-axis is strongly suppressed (Yehuda et al., 1995a,b), CRH levels in CSF are increased (Bremner et al., 1997a,b; Kasckow et al., 2001b). Finally, although the HPA-axis is generally activated in Alzheimer’s disease (Fig. 5), some authors have reported decreased CRH levels in the CSF of these patients (Gottfries et al., 1995; Geriacioti et al., 1992), although others could not confirm this (Valenti, 1996; Banki et al., 1992; Nemeroff, 1996; Martignoni et al., 1990). In conclusion, alterations in HPA-axis activity are not likely to be directly reflected by CRH levels in the CSF.

An important argument for a crucial role for CRH in depression is that the symptoms associated with and in fact closely resembling depression, such as decreased food intake, decreased sexual activity, disturbed sleep and motor behavior and an increased anxiety, can all be induced in experimental animals by intracerebroventricular injection of CRH (Holsboer et al., 1992). In addition, antidepressant drugs attenuate the synthesis of CRH by stimulation and/or upregulation of corticosteroid receptor expression (Reus et al., 1997; Fischer et al., 1990; Brady et al., 1991, 1992; Delbende et al., 1991; Reul et al., 1993; Nemeroff, 1996; Yau et al., 1995, 2002; Pariante and Miller, 2001; Pariante et al., 2003). Moreover, the CRH concentrations in CSF in healthy volunteers (Veith et al., 1993) and of depressed patients (De Bellis et al., 1993; Heuser et al., 1998) decrease due to antidepressant drugs. However, as argued before, CSF-CRH is also derived from other sources, such as the cortex (Vythilingam et al., 2000; see also before). Lastly, a transgenic mouse model with an overproduction of CRH appeared to have increased anxiogenic behavior, i.e., symptoms that are usually related with major depression, that could be counteracted by injection of CRH antagonist (Stenzel-Poore et al., 1994). CRH-receptor antagonists may be useful for the treatment of melancholic depression (Grammatopoulos and Chrousos, 2002). A mouse with a genetic deletion of the CRH-1 receptor has reduced anxiety-like behavior (Contarino et al., 1999). An interesting new compound in depression research is urocortin, a CRH-related peptide that also exerts anxiogenic-like properties in experimental animals (Moreau et al., 1997; Behan et al., 1997). The presence of a CRH-binding protein in the brain that also binds urocortin and thus could allow modulation of its bioavailability is particularly interesting in this respect (Behan et al., 1997).

Together, among other things, the arguments mentioned above have led to the CRH-hypothesis of depression, i.e., hyperactivity of a subgroup of CRH neurons that do not project to the median eminence but into the brain, but that are activated in depression and induce the symptoms of this disorder. The recent development of selective small molecule
CRH1 receptor antagonists, which block both the effects of CRH in vitro and in vivo, suggests that these compounds could be effective in the treatment of mood and anxiety disorders (O’Brien et al., 2001b). In an open trial, one of such compounds (R121919) led to a 50% reduction in depressive symptoms, comparable to selective serotonin re-uptake inhibitor treatment (Keck and Holsboer, 2001).

There are, however, also observations that may call this concept into question and point rather to a causal role for glucocorticoids (Fig. 7). A few studies have reported that glucocorticoid (receptor) antagonists may be effective in the treatment of major depression (Murphy, 1997; Wolkowitz et al., 1999). Inhibitors of cortisol production such as metyrapone, aminoglutethamide or ketoconazole, when administered to major depressed patients, may result in clinical success, which is not to be expected if an increase in CRH itself would cause the symptoms (Fava, 1994; Reuss, 1977; Murphy, 1997). The anti-glucocorticoids dehydroepiandrosterone (DHEA) and DHEA-sulphate are also studied for their positive anti-depressant and cognition enhancing effects in mood disorders (Reus et al., 1997; Wolkowitz et al., 1997). The glucocorticoid receptor antagonist mifepristone (RU486) is effective in treating psychotic depression, producing clinically relevant responses at high dosages in considerable numbers of patients within a few days of treatment (Gold et al., 2002; Belanoff et al., 2002); a clear contrast with most classic antidepressants that generally need at least a month to become effective. However, these compounds induce so many unspecific effects, also at the central level, that the interpretation allowed by some of these experiments is limited (Holsboer and Barden, 1996). On the other hand, the observation that a significant improvement in mood occurred in patients with treatment-resistant depression who received dexamethasone while remaining on their antidepressant (sertraline or fluoxetine) treatment supports the concept of hyperactive CRH neurons playing a causal role in the symptomatology of depression (Dinan et al., 1997).

The increased drive of the HPA-axis in depressed patients could be mediated either by CRH, by vasopressin, or by both. Indeed, CRH receptor antagonists, cortisol synthesis inhibitors, or corticosteroid receptor antagonists may be effective in depressed patients (Holsboer, 2000; Gold et al., 2002). Whether disinhibition of sexual behavior is a side effect of CRH-antagonists in depressed patients as was observed following intracerebroventricular administration in animals (Jones et al., 2002) is not yet known. Gold et al. (1995) and Gold and Chrousos (2002) propose that the classic form of major depression, i.e. “melancholic depression”, that goes with decreased food intake, insomnia, lack of affective responses to external events, and a general state of pathological hyperarousal, would be due to a hyperactivity of CRH neurons. Hypercortisolism is consistently observed in melancholy. The adrenal is hyperresponsive to ACTH and hypertrophic, while the pituitary cells are appropriately responsive to glucocorticoids. In contrast, “atypical depression”—a state of hyperphagia, hypersomnia, enhanced affected responsiveness to external stimuli, lethargy and fatigue would be based upon increased levels of corticosteroids and decreased levels of CRH (Gold et al., 1995).

In this context it is of interest that women are not only more at risk than men for major depression, but also have a significantly higher glucocorticoid and mineralocorticoid receptor mRNA expression than men in the temporal lobe and prefrontal cortex (Watzka et al., 2000). In addition, a well-known side effect of glucocorticoid treatment is...
depression, and a third of the patients receiving glucocorticoids experience significant mood disturbances and sleep disruption. Up to 20% report psychiatric disorders, including depression, mania, and psychosis (Mitchell and O’Keane, 1998). Moreover, atypical depression is found in a large proportion of the patients with Cushing’s disease. Especially patients with longer duration of Cushing’s syndrome are at risk for such psychopathology (Gold et al., 1995; Dorn et al., 1995). The fact that atypical depression is so often seen in Cushing’s syndrome indicates that in these patients elevated cortisol levels produced by the tumor causes this type of depression rather than ACTH or CRH. This conclusion is supported by a small study that showed that depression can be treated by ketoconazole, an antiglucocorticoid (Wolkowitz et al., 1999), and by the observation that metyrapone successfully treats depression in Cushing patients (Checkley, 1996). However, it should be noted that also after correction of the hypercortisolism in Cushing’s syndrome, atypical depression frequently continues to be present and suicidal ideation and panic may even increase (Dorn et al., 1997). Despite the pronounced hypercortisolism, the pituitary shows a profoundly exaggerated plasma ACTH response to CRH in Cushing’s disease, indicating that the pituitary is grossly unresponsive to glucocorticoid negative feedback. A relationship has also been found between hypercortisolism and violent suicidal behavior. Increased urinary cortisol excretion and a decreased noradrenergic function were present in patients who recently attempted suicide and in those with a history of suicidal behavior (Van Heeringen et al., 2000). Also patients suffering from a winter depression or chronic fatigue syndrome meet all the criteria for an atypical depression. In these patients, hypofunctional CRH neurons and an enhanced glucocorticoid feedback sensitivity are postulated (Gold et al., 1995; Joseph-Vanderpool et al., 1991; Visser et al., 2001), but so far the hypothalamus of such patients has not been investigated. In multiple sclerosis (MS), a disease with an increased incidence of depression, the blunted plasma ACTH response to vasopressin reflects a relative shift from CRH to vasopressin modulation of pituitary adrenal function (Gold et al., 1995), which fits in with the increased numbers of neurons colocalizing AVP and CRH in this disorder (Erkut et al., 1995).

The consistent increase in HPA-axis activity in many depressed cases raises the question of the possible underlying pathogenic mechanisms. At least in animal models, antidepressant treatment normalizes changes in glucocorticoid levels (Yau et al., 1995, 2002). So far, an imbalance in the ratio between MR and GR has been shown in depressed patients (Young et al., 2003), but it is not clear whether this is cause or effect of the disorder. Impaired negative feedback control of the HPA-axis and adrenal hypertrophy are commonly found in a subgroup of depressed patients (Checkley, 1996; Modell et al., 1997). They coincide with episodes of depression and reverse, at least partially, after recovery from psychopathology. Some observations suggest that the impaired negative corticosteroid feedback on the HPA-axis in a number of healthy probands at risk for affective disorder is caused by a disturbed corticosteroid receptor function, indicating a genetically transmitted risk factor (Holsboer et al., 1995; Holsboer, 2000). Genetic variations (polymorphisms) of the glucocorticoid receptor may explain why only some 50% of the depressed patients show hypercortisolaemia and why considerable variation in their symptoms occurs (Checkley, 1996; Holsboer, 2000). However, so far the expected mutations, deletions and other changes in the glucocorticoid receptor gene that were predicted to be responsible for a relative glucocorticoid resistant state in considerable
numbers of patients (De Rijk et al., 2003) have not yet been found (Brönnegård et al., 1996; Holsboer, 2000; De Rijk et al., 2003).

An alternative possibility involves changes during early development that can induce altered feedback control of the HPA-axis that may persist into adulthood, and could lead to acquired glucocorticoid receptor resistance in specific feedback areas (De Bellis et al., 1999; Brönnegård et al., 1996; De Kloet et al., 1997) and GR hypersensitivity in other brain regions (Nemeroff, 1996). Several developmental sequelae might be considered in this respect. Pharmacological interference but also early stressors have been implicated. Depression and anxiety have been found to be more frequent in the sons and daughters of women who had been treated with DES during pregnancy (Vessey et al., 1983; Brown et al., 1995b) and in children of mothers who were pregnant during the hunger winter in the Netherlands during the second world war (Susser and Lin, 1992; Susser et al., 1996). However, so far the HPA-axis has not been investigated in these patients. In addition, (psychological) stress during development may have permanent activating effects on the HPA-axis (see Meaney et al., 1996 and before; Weaver et al., 2004; Caldji et al., 2000; Francis et al., 1999).

1.7. Sex hormones, in depression, premenstrual syndrome, antepartum depression, postpartum depression and menopausal mood disorder

There is a close functional interaction between the HPA-axis and the HPG-axis. Hypothalamic CRH neurons inhibit directly or indirectly through, for example, proopiomelanocortin neurons, the hypothalamic control of the gonadal axis (Dudas and Merchenthaler, 2002a; Rivest and Rivier, 1995). In addition, glucocorticoids secreted from the adrenal cortex may act on the HPG-axis at different levels. On the other hand, estrogens derived from the ovaries stimulate the HPA-axis through an interaction of estrogen receptors (ERs) via specific estrogen-responsive elements (EREs) in the promoter region of the human CRH gene (Vamvakopoulos and Chrousos, 1993). Pregnant women as well as women receiving high-dose estrogen therapy have elevated levels of free cortisol both in morning and evening plasma samples during pregnancy and estrogen-gestagon treatment (Lindholm and Schultz-Moller, 1973), while estradiol can also downregulate glucocorticoid receptors in the anterior pituitary, hypothalamus and hippocampus. The latter will interfere with glucocorticoid negative feedback and will consequently increase the activity of the HPA-axis (Paulmyer-Lacroix et al., 1996). A variety of studies have further indicated the involvement of estrogens and androgens in mood and affective disorders.

Unipolar depression and dysthymia are twice as common in women as they are in men (Piccinelli and Wilkinson, 2000; Seeman, 1997), while women have a two-fold-higher lifetime prevalence of major depression than men (Lehtinen and Joukamaa, 1994; Pearlstein et al., 1997). Both observations point to an organizing or an activating effect of sex hormones in the pathogenesis of depression or to a combination of both. The HPA-axis is highly susceptible to neonatal programming (Plotsky and Meaney, 1993; Shanks et al., 2000; Viau et al., 1993). In rodents, early androgen exposure disrupts feminine behavior, gonadotropin release and neuroanatomical sexual differentiation (Barraclough and Gorski, 1961; Hary et al., 1986) and suppresses the HPA-axis (Seale et al., 2004), while in humans...
prenatal estrogen administration (e.g., DES) was found to increase the risk of affective disorders (Meyer-Bahlburg and Ehrhardt, 1986, 1987).

Recently, we found the number of CRH neurons to be significantly larger in patients with mood disorders than in controls. Moreover, the number of CRH neurons colocalizing ERα increased proportionally in mood disorders (Bao et al., 2005). Since the HPA-axis is considered to be the ‘final common pathway’, both in the normal stress response and in the depressive symptomatology (Holsboer and Barden, 1996; Swaab et al., 2000), these observations raise the possibility that sex steroids are involved in prenatal programming and postnatal activation of the CRH neurons, thus influencing gender-related differences of the risk of mood disorders and the stress response (Bingaman et al., 1994; El Hani et al., 1980; Handa et al., 1994).

Variations in the sex hormone levels during a woman’s reproductive years are bound to affect the stress system as well. The stress system receives intermittent positive input from estradiol that increases during puberty. In addition it also interacts with androgens, which may contribute to an increased vulnerability to disorders or states characterized by aberrant CRH secretion, such as melancholic and atypical depression, eating disorders, chronic active alcoholism or other addictions, seasonal affective disorder, the chronic fatigue and fibromyalgia syndromes, and several autoimmune disorders (Chrousos, 1992; Young and Altemus, 2004). Moreover, the prevalence of major depression increases during the reproductive years, especially during times when sex hormone levels show rapid fluctuations, such as in the premenstrual, antepartum and postpartum periods, and during the transition phase to the menopause (Lehtinen and Joukamaa, 1994; Paykel, 1991; Pearlstein et al., 1997; Young and Korszun, 2002). Depression can also be associated with the use of oral contraceptives, pregnancy and menopause (Parry and Newton, 2001) (see below). Interestingly, as early as 1919, M. Bleuer suggested that hormone treatment could be a potential antidepressant (Holsboer and Barden, 1996), while there is indeed some evidence that hormone replacement therapy could improve and prevent postpartum depression (Gregoire et al., 1996; Sichel et al., 1995; Young et al., 2000).

In women with depression, plasma levels of estradiol are often significantly lower, which might be due to inhibition of the reproductive axis by the HPA-axis, comparable to the situation during stress or after CRH administration (Young et al., 2000). Not only is estradiol lower in depressed women, LH pulsatility, too, is slower and dysrhythmic (Meller et al., 2001). It should be noted, however, that there is evidence showing depressed female patients had significantly higher amplitudes and some higher 24 h mean levels of diurnal estradiol rhythms than controls and the change of reproductive hormones is speculated to play a more important role than the absolute level of the sex hormones in the etiology of depression or in the vulnerability to this disorder (Bao et al., 2004). The drop in estrogen levels in postmenopausal women may be a factor in both the pathogenesis of late-life depression and in the response to estrogen replacement therapy. In untreated depressed female patients, on the other hand, significantly higher plasma concentrations of testosterone, androstenedione and dehydrotestosterone were found, which may result from overstimulated adrenal glands in the hypercortisolaemic depressed patients (Weber et al., 2000).

In contrast to women, depressed men generally show decreased testosterone levels. In males, testosterone levels were lower in severely depressed patients (Heuser, 2002), and
older men with lower bioavailable testosterone levels were found to be more frequently depressed (Barrett-Connor et al., 1999a,b). Low testosterone levels were also found in men with dysthymic disorder (Seidman et al., 2002). The difference in androgen levels between depressed women and men may be explained by an activation of the adrenal and an inhibition of the HPG-axis in both, taking into consideration the fact that half of the circulating testosterone of healthy young menstruating women is derived from ovarian secretion (Abraham and Carpenter, 1997) while the other half is from the adrenal (Vermeulen and Ando, 1978). The activation of the HPA-axis in depressed men may negatively affect gonadal function at every level of regulation, similar to females (Albert et al., 1993; Baischer et al., 1995; Schweiger et al., 1999; Sternbach, 1998; Weber et al., 2000). It should be noticed, however, that the sex-opposing effects of androgens and estrogens on pituitary-adrenal function were found in nonpregnant primates (Giussani et al., 2000). So far, the situation in humans remains unclear in this respect.

Both the testosterone level and androgen receptor polymorphism are related to the risk for middle-aged men to become depressed. Men who have low total testosterone levels and a shorter CAG codon repeat length in the androgen receptor have a greater likelihood of becoming depressed (Seidman et al., 2001). In connection with the observed decreased sex hormone levels in depressed men, it is interesting that in bodybuilders who take supraphysiological doses of testosterone, testosterone levels had a strong negative correlation with depression scores (Dickerman and McCobathy, 1997). Studies in anabolic androgenic steroid users show that some of them develop manic or aggressive reactions to these drugs. Supraphysiological doses of testosterone indeed increased ratings of manic symptoms in normal men (Pope et al., 2000). Moreover, estrogens can induce hyperresponsiveness of the HPA-axis to stimuli in normal men; an effect that seems to be due to estrogen rather than to other female-specific factors (Kirschbaum et al., 1996).

Similarly, the plasma norepinephrine response in these men was augmented by estrogen, possibly because of the stimulation of CRH neurons (which innervate and stimulate central noradrenergic neurons) or because of direct effects on the production or metabolism of norepinephrine (Spies et al., 1997; Zukowska-Grojec et al., 1991).

So far the exact mechanism of the sex hormones’ involvement in mood or affective diseases remains unclear. Apart from the effects on the HPA-axis, an interaction between sex hormones and the serotonergic system has been proposed (Bloch et al., 1998). We recently found that not only estrogen receptor (ERα), but also androgen receptor is colocalized with CRH in the human PVN, and a similar pattern of up-regulation of CRH-nuclear ERα was observed in males and females in depression (Bao et al., 2005). It is known that the CRH promoter contains estrogen-responsive elements (EREs) (Vamvakopoulos and Chrousos, 1993). Recently, we have shown that androgen-responsive elements (ARE) are present in the human CRH gene promoter region (Bao et al., 2005). The effects of estrogens and androgens on human CRH neurons may thus well be direct ones. Whether circulating sex steroids (Lindholm and Schultz-Moller, 1973) or local production of estrogens, involving aromatase and adrenal or brain-derived androgens, may act as a ligand and up-regulate ERα in the brain (Kroboth et al., 1999), or whether up-regulation of ERα is modulated by neurotransmitters, such as norepinephrine, dopamine or serotonin (Asberg et al., 1976; Blaustein et al., 1986), is unknown and deserves further study in human. An interesting finding in this respect is the changing neurosteroid levels in
plasma during depression (Romeo et al., 1998), indicating that central or peripheral metabolites of steroid hormones, which are capable of affecting many systems in the brain, are involved.

Both sexual function, and mood improved in hypogonadal men that received testosterone replacement (Fink et al., 1998, 1999; Seidman and Walsh, 1999; Wang et al., 1996; Wong et al., 2000). However, further controlled studies will be required to establish that testosterone administration is effective in mood disorders (Sternbach, 1998). Estrogen replacement therapy may make women with Alzheimer’s disease less vulnerable to depression (Carlson et al., 2000) and may augment fluoxetine response in elderly depressed patients (Schneider et al., 1997). On the other hand, it should be noted that estrogen substitution in postmenopausal women with depressive symptoms was effective in some studies but not in others (Rasgon et al., 2001; Rubinow et al., 1998). Progestins during sequential hormonal replacement therapy cause negative mood and physical symptoms that are accentuated by increasing the estrogen dose (Björn et al., 2003).

1.8. Premenstrual syndrome or premenstrual dysphoric disorder

During the reproductive years, a woman is exposed to a monthly fluctuation of circulating estradiol and progesterone that may affect her behavior, mood, and immune system and other functions. The monthly fluctuations of estradiol that accompany menstrual cycles are expected to influence the secretion of central nervous system CRH and catecholamines until menopause. Premenstrual syndrome, or premenstrual dysphoric disorder, is characterized by depression, anxiety and mood swings during the last week of the luteal phase, with decreased secretion of CRH and an increased incidence of suicides and enhanced vulnerability to autoimmune and allergic inflammatory phenomena (Fourestie et al., 1986; Hayward et al., 1997; Rabin et al., 1990; Skobeloff et al., 1996). It has been found that the time of the maximum emotional disturbance coincides with the decrease of plasma estradiol levels during the latter part of the luteal phase of the cycle, when plasma progesterone levels are still elevated (Hayward et al., 1997). Other studies have suggested that the period of peak estradiol secretion in the state immediately before ovulation is associated with elevations in mood, a phenomenon that might contribute to fecundity (Blum et al., 2004; Davydov et al., 2004; Endicott, 1993; Henderson and Whissell, 1997).

Correlations have further been reported between the premenstrual or menstrual phase and violent crimes, death as a result of accident or suicide, accidents, admission to hospital with psychiatric problems, taking a child to a medical clinic, and loss of control of aircraft and plane crashes in which the women pilots were said to be menstruating at the time of the crash (Parlee, 1973). A polymorphism in the serotonin transporter promoter gene region may be a vulnerability factor for premenstrual dysphoric syndrome (Praschak-Rieder et al., 2002). This syndrome is characterized by disturbances in the timing and secretion patterns of circadian rhythms and their response to critically timed light administration, and interventions with bright light improves mood in these patients (Parry and Newton, 2001). Although there is at present no conclusive evidence that premenstrual dysphoric disorder is indeed associated with abnormalities in the levels of sex hormones, both suppression of
ovarian function by LHRH agonists and surgical ophorectomy are effective treatments for this type of mood disorder (Rubinow et al., 1998; Steiner, 1996).

The observation that no differences were present in plasma levels of ACTH, β-endorphin, cortisol or free testosterone does not support a primary HPA-axis abnormality in women with premenstrual syndrome (Bloch et al., 1998). Timing rather than quantitative measures of cortisol secretion were different in premenstrual dysphoric subjects, both during the menstrual cycle and in response to sleep deprivation interventions (Parry et al., 2000). Moreover, on the basis of animal experiments, neurosteroids have been proposed as potential etiological factors in this syndrome (Britton and Koob, 1998). Notably, such effects would not be reflected in peripheral hormone changes. The fluid retention in the premenstrual syndrome may be related to increased vasopressin levels (Ishunina et al., 1999a,b; Ishunina and Swaab, 1999; Reid and Yen, 1981). This peptide indeed shows fluctuation during the menstrual cycle, but the relationship between these fluctuations and psychological symptoms of the premenstrual syndrome has not been shown as yet. Sleep deprivation may help to correct underlying circadian rhythm disturbances during sleep in premenstrual dysphoric disorder (Parry et al., 1999).

1.9. Antepartum depression

Antepartum depression is found in some 5% of pregnant women (Campagne, 2004). This condition may be a risk factor for the development of preeclampsia and is the strongest predictor of postpartum depression. Maternal depressive symptoms during pregnancy may lead to behavioral changes in the child (Oren et al., 2002a,b). The safety of pharmacological treatment of depression in pregnant women is controversial because of the possible behavioral-teratological effects (Swaab, 1972). It is therefore of great practical interest that an open trial showed that morning light therapy may be effective as an antidepressant during pregnancy (Oren et al., 2002a,b).

The second half of the human pregnancy term is associated with hypercortisolism. Indeed, the levels of free plasma cortisol and 24-h urinary free cortisol excretion in pregnancy overlap with levels in patients with mild Cushing syndrome (Nolten et al., 1980). Placental CRH causes this hypercortisolism of human pregnancy. By 28–30 weeks of gestation, CRH levels in plasma are similar to those in the portal system, whereas the levels of CRH-binding protein are similar to those in nonpregnant women and normal men (Challis, 1995; Linton et al., 1993). Anoxia, inflammatory cytokines, several prostaglandins, and glucocorticoids themselves cause placental CRH secretion in vitro and in vivo. This means that sustained anoxia caused by preeclampsia or eclampsia, increases of circulating cytokine levels caused by infection or inflammation, and increased glucocorticoid concentrations caused by physical or emotional stress may all initiate premature labor through increases in CRH secretion. The increased levels of glucocorticoids under these conditions may also interfere with mood.

1.10. Postpartum mood disorders

The postpartum period is characterized by an increased incidence of psychiatric and autoimmune manifestations. The ‘postpartum blues’, a mild form of transient depression,
occurs in 60–70% of women; full-blown postpartum depression affects about 10% and very severe postpartum psychosis affects about 1 in 1000 (Affonso and Domino, 1984). In addition, autoimmune diseases, such as ‘postpartum thyroiditis’ and rheumatoid arthritis, frequently develop or are acutely exacerbated during the first few months postpartum (Amino et al., 1982, 1978; Iijima et al., 1998; Stagnaro-Green, 2004).

In the commonly occurring postpartum mood disorders depression and psychosis, gonadal hormones have often been presumed to be of pathogenetic importance. The postpartum estrogen withdrawal state has often been held responsible for this disorder. However, available studies show a lack of evidence that serum sex hormones account for mood disturbances in these women, although there is evidence that estradiol might be effective in its treatment (Gregoire et al., 1996; Sichel et al., 1995). Clinical implications of placental CRH extend beyond pregnancy, labor, and delivery (Magiakou et al., 1996b; McLean et al., 1995). It has been noticed that, although several depressive conditions such as melancholic depression and anorexia nervosa are typically associated with high hypothalamic CRH secretion, atypical or seasonal depression, chronic fatigue and fibromyalgia syndromes, and the Cushing syndrome before and during the first year after surgery, are all associated with a decreased production of hypothalamic CRH (Chrousos, 1992; Dorn et al., 1995; Dorn and Chrousos, 1997; Magiakou et al., 1996a).

There is also evidence showing that the postpartum period might be associated with low hypothalamic CRH secretion, which would predispose patients to atypical depression and autoimmune phenomena (Magiakou et al., 1996a). It is supposed that in the postpartum state, the major source of CRH and estrogen, the placenta, is no longer present, whereas the hypothalamic CRH production is probably suppressed as a result of previous exposure to high levels of cortisol and because of concurrent estrogen deficiency. High-dose estrogen has a marked antidepressant effect during this time, possibly because it reestablishes normal stress system secretion of CRH and norepinephrine (Gregoire et al., 1996). There is also evidence showing that a hyperactive HPA-axis in the third trimester of human pregnancy is driven, at least partly, by progressively increasing circulating levels of CRH-binding protein. The central suppression of postpartum hypothalamic CRH secretion is presumed to cause an increased vulnerability to affective disorders, as they are frequently observed during this period. The suppressed ACTH response to ovine CRH may serve as a biochemical marker of the ‘postpartum blues’ or depression (Magiakou et al., 1996a).

The hypothesis that the postpartum drop in melatonin secretion would be responsible for this disorder (Sandyk, 1992a,b) has so far not been supported by clinical evidence. On the other hand, two women, both suffering from a major depressive episode with postpartum onset, were effectively treated with bright light (Corral et al., 2000), which affects the HPA-axis via the SCN. Alterations in the HPA-axis that can be attributed to childbearing show remarkable similarity to those observed in depressed women. Postpartum women are also at risk for hypothalamic-pituitary-thyroid-axis dysfunction that may increase the vulnerability for affective disorders (Sichel et al., 1995; Wisner and Stowe, 1997).

1.11. Menopausal mood disorder

Depressive symptoms are common during the transition to menopause, and there are suggestive data that estrogen deficiency may increase the susceptibility to depression
(Birkhauser, 2002). However, although perimenopause may be a period of risk for mood disturbances, it generally does not lead to major depression. In fact, according to some studies, depressive disorders do not occur more frequently during perimenopause (Banger, 2002).

There is therefore no conclusive evidence of an HPA-axis dysfunction during the perimenopausal period, even though studies have shown that the morning plasma cortisol levels are decreased by about 40–50% during the perimenopausal and early menopausal period (Ballinger, 1990). These studies suggest that estrogen withdrawal took place during these periods and was probably accompanied only by transient hypoactivation of the stress system. Women with both of these estrogen and, hence, CRH withdrawal states, do show mood improvements with estrogen therapy (Soares et al., 2001). Estrogen replacement therapy also improves mood in postmenopausal women and estrogens may improve the effect of SSRIs in postmenopausal women (Birkhauser, 2002).

1.12. The glucocorticoid cascade hypothesis: brain damage?

In depression, the HPA-axis is strongly activated and the adrenal cortex hypersecretes glucocorticoids. Although less pronounced, a considerable HPA-activation is nevertheless also found in multiple sclerosis, anorexia nervosa, schizophrenia and AD. When a stressor triggers the production of CRH, vasopressin and possibly other ACTH secretagogues stimulate ACTH release, which subsequently triggers cortisol release from the adrenals. In addition, the adrenals are stimulated by autonomic nervous pathways arising in the hypothalamus (Buijs and Kalsbeek, 2001). Cortisol, and synthetic corticosteroids inhibit the release of CRH, vasopressin (Erkut et al., 1998, 2002) and ACTH. Cortisol also acts on other parts of the brain, that regulate cardiovascular tone and inflammatory adaptive responses. In humans we observed a gradual hyperactivation of CRH neurons with age (Raadsheer et al., 1993, 1994a; Figs. 2 and 3) which is in full agreement with hormone assays that indicate a general activation of various HPA-axis parameters during the course of human aging. Basal corticosterone levels in rats generally remain unaltered with aging, although stress responses in old animals and the duration of recovery after stress generally takes more time, but considerable differences exist between strains (Lucassen and De Kloet, 2001; Heine et al., 2004b).

On the basis of earlier animal experiments, overexposure to glucocorticoids during prolonged periods of stress was expected to be damaging to the brain, especially in aged animals, and particularly affecting the hippocampus. In a series of studies in rats, Landfield et al. (1981) produced experimental evidence demonstrating that cumulative exposure to corticosteroids influences hippocampal neuronal viability as well as function, which was expected to seriously compromise memory function and cognition. Subsequently, Sapolsky et al. (1986) and Sapolsky and McEwen (1986) provided evidence that chronic stress, with its ensuing increase in corticosteroid levels, caused degenerative loss of, pyramidal, neurons in the hippocampus and subsequent deficits in memory function and cognition in rats. Furthermore, persistently elevated GC or dexamethasone exposure was reported to induce reactive glial cell proliferation, reduced dendritic branching in the CA3 area as well as reductions in volume, and reduced cell numbers in CA1 and CA3 (Landfield et al., 1978, 1981; Sapolsky et al., 1985, 1990; Sapolsky, 1985, 1986, 1996, 1999; Haynes...
et al., 2001; Lee et al., 2002). Chronic exposure for 6 months to inescapable foot shock stress for 4 h per day resulted in endogenous hypercortisolism and even induced CA1 pyramidal neuronal loss, but only in senescent rats (Kerr et al., 1991). However, these older studies on high GC exposure, stress and hippocampal viability generally applied either rather extreme, often psychosocial stressors, or pharmacologically high GC concentrations (Sapolsky et al., 1985; Sapolsky, 1996, 1999). Studies performed under less extreme, more physiologically relevant social stressors, or e.g., in non-rodent and primate species, have so far provided conflicting data (see below).

One of the first premises at the time was that the rat hippocampus with its high density of mineralocorticoid as well as glucocorticoid receptors, was thought to inhibit CRH activity directly. The tonic inhibitory control on HPA-axis activity (see above and: Ratka et al., 1989; Herman et al., 1989; Sapolsky et al., 1991; Jacobson and Sapolsky, 1991) is now known to be exerted through several, often indirect neural pathways (Herman and Cullinan, 1997), while GC feedback of the HPA-axis takes place primarily at the level of the hypothalamus and pituitary (Kretz et al., 1999). However, damage to the hippocampus was proposed to cause a disinhibition of the glucocorticoid negative feedback, which would lead to a further activation of the HPA-axis, to subsequent rises in glucocorticoid levels and thus to accumulating damage to the hippocampus. This hypothetical feed-forward cascade became known as the “glucocorticoid cascade hypothesis” and was proposed as a major pathogenetic mechanism also in other human neurodegenerative diseases associated with HPA-axis alterations. Since the HPA-axis is indeed activated in AD and depression, a glucocorticoid cascade was proposed to be causally involved in hippocampal damage, particularly in these disorders (Sapolsky et al., 1986; Sapolsky and McEwen, 1986). Excessive, repetitive hypersecretion of cortisol, and possibly even the increased basal levels of the hormone, were supposed to accelerate the course of hippocampal damage in AD (Sapolsky et al., 1986; Lee et al., 2002).

Increased cortisol levels would not only cause hippocampal neuronal damage but was also proposed to potentiate Aβ-amyloid toxicity. Conversely, DHEA and its sulphate DHEAS are believed to exert a neuroprotective action (Murialdo et al., 2000). Many AD patients indeed present with a non-suppression of plasma cortisol following dexamethasone administration (Swaab et al., 1994, 1995b; O’Brien et al., 1996) and the degree of hyperactivity of the HPA-axis generally correlates with the severity of cognitive impairment and hippocampal atrophy (Gurevich et al., 1990; De Leon et al., 1988; Weiner et al., 1997; Lupien et al., 1998). An alternative and likely explanation for the latter observation is, however, the possibility that cortisol does not play a causal role, but that both the activation of the HPA-axis and impaired cognition are explained by the ongoing AD process, which occurs relatively early in the hippocampal area.

Other observations in various disorders, too, only partly agree with the glucocorticoid cascade hypothesis. It is a well-established fact that in the absence of concomitant stress exposure, glucocorticoid treatment causes memory disturbances (Lupien and McEwen, 1997; Lupien et al., 1998; Wolf, 2003; Newcomer et al., 1994). Patients receiving chronic corticosteroid therapy were found to have smaller hippocampal volumes, lower N-acetyl aspartate ratios, and declarative memory deficits compared with controls (Brown et al., 2004). A dosage of 5–40 mg of prednisone daily for at least 1 year caused patients to perform worse than controls on hippocampal-dependent tests of explicit memory, while the
groups did not differ with respect to the hippocampus-independent implicit memory task. Elderly patients were more susceptible to memory impairment with less protracted treatment. Even acute treatment with prednisone can adversely affect memory. In addition, depressed patients who did not suppress cortisol when given dexamethasone made more mistakes in a verbal memory task (Wolkowitz et al., 1990). There is, therefore, a possibility that the proposed potential benefit of anti-inflammatory treatment of AD patients with synthetic glucocorticoids (Aisen and Pasinetti, 1998) may be counterbalanced by the memory impairment of these compounds. Some data suggest, moreover, that nonsteroidal anti-inflammatory drugs exert a stronger protective influence than steroids (Breitner, 1996). It is, however, surprising that patients with systemic lupus erythematosus, who do not have an overt neuropsychiatric disease, show improved cognitive performance after treatment with prednisone (Aisen and Pasinetti, 1998). Moreover, in contrast to the often claimed association between elevated cortisol levels and impaired declarative memory performance, subjects with a remarkably high increase in cortisol levels in response to psychological stress appeared to show improved memory performance (Domes et al., 2002).

The presumed mechanisms for hippocampal damage by corticosteroids, based upon animal experiments, are supposed to be inhibition of glucose transport into hippocampal neurons and glia, the modulation of long term potentiation and primed burst potentiation by glucocorticoids (Joëls et al., 2004), and the potentiation of the effects of excitatory amino acids that might kill hippocampal neurons. Cortisol injection reduces hippocampal glucose metabolism in normal elderly people, but not in AD (De Leon et al., 1997). The hippocampal insensitivity to cortisol in AD can, in fact, be seen as an argument against the glucocorticoid cascade hypothesis for this disorder (see below). Although cell death is often presumed to be the mechanism behind cerebral atrophy following prednisone administration, this idea is not consistent with histological and neuropathological examination of patients that suffered from depression or were exposed to synthetic corticosteroids. We could not find any support for this presumption in postmortem material of these patients (Lucassen et al., 2001a; Müller et al., 2001) and failed to find any obvious neuropathology or significant structural, synaptic, or AD-like alterations in the hippocampus using Alz-50, glial fibrillary acidic protein, Nissl, Bodian Silver stains, or with synaptophysin or B-50, markers for synaptic density and plasticity. Moreover, hippocampal volume reductions in Cushing’s disease were shown to be reversible after a decrease or cessation of the steroid exposure. This agrees with the general clinical experience with depressive or Cushing’s patients, in which treatment or operation can relieve the depressive symptoms, several of the HPA alterations, and even the hippocampal atrophy (Starkman et al., 1999; Lucassen et al., 2001a; Müller et al., 2001). Regarding the discrepancy between hippocampal volume reductions in the absence of obvious changes in neuron number, a possibility could be a shift in water content (Bentson et al., 1978). In children with intractable epilepsy, ACTH-induced brain shrinkage was more remarkable in subjects under 2 years of age. Brain size as visualized by imaging almost returned to its original status in seven out of nine cases that were followed for between 1 and 3 months after therapy. In adult long-term steroid users cerebral atrophy may occur, which can improve following decrease or cessation of steroid use. Brain shrinkage seemed thus to be due mainly to changes in water and electrolyte content (Bentson et al., 1978; Satoh et al., 1982; Krishnan et al., 1991).
Also in Cushing’s syndrome a high incidence of cerebral and cerebellar atrophy and cognitive dysfunction has been reported. Significant positive correlations were observed between the size of the hippocampal formation and memory tests. Negative correlations were seen with plasma cortisol levels in Cushing’s patients (Starkman et al., 1992). In Cushing’s disease, decreased cerebral glucose metabolism has been observed that may contribute to the cognitive and psychiatric abnormalities in Cushing’s disease (Brunetti et al., 1998). There is a remarkable discrepancy between the relatively intact neurological and psychiatric status of most patients that are treated with glucocorticoids and the obvious ventricular and sulcal enlargement of their brains. In addition, at least partial recovery of the brain atrophy may occur following cessation of corticosteroid administration (Benton et al., 1978; Yehuda, 1997; McEwen, 1997; Starkman et al., 1999; Bourdeau et al., 2002; Vythilingam et al., 2002), arguing against the induction of massive, irreversible hippocampal cell loss as a mechanism. This means that the cerebral atrophy observed during hypercortisolaemia cannot simply be compared to that found in AD. Loss of brain volume induced by glucocorticoids might be due to a loss of water as indicated by MRI in depression (see above; Krishnan et al., 1991).

In depression the glucocorticoid cascade has also been presumed to result in damage of the hippocampus (Sapolsky and McEwen, 1986). Cognitive impairment can persist after recovery from depression, particularly in the elderly, although not always (Sachs and Shulman, 2005). However, brain atrophy in depression is much less pronounced than in Cushing’s disease, if present at all (Abas et al., 1990; Vakili et al., 2000). One study did not observe any differences between the hippocampal volumes of depressed patients and controls as determined by MRI. In addition, dexamethasone suppressors and non-suppressors did not differ in hippocampal volume (Axelson et al., 1993), raising doubts about the relationship with cortisol levels. In a later study, decreased hippocampal volumes were found in female patients with a history of recurrent major depression, while cerebral volumes were not different from controls, and in a group of aged female early-onset subjects with depression where right hippocampal volume was found to be reduced, independent of the extent of subcortical white matter lesions (Janssen et al., 2004). Clearly, part of the discrepancies with former studies may involve lateralization, as Bremner et al. (2000) found only a volume decrease in the left hippocampus. In addition, the higher spatial resolution achieved in more recent studies is of importance when hippocampal gray matter was assessed exclusively. These patients were not suffering from depression at that particular moment, and therefore not likely to be affected by the acute effects of corticosteroids. Furthermore, differences in disease duration should be taken into account (Sheline et al., 1999, 2003), while also other (methodological) issues are possibly involved (Sapolsky, 2001; Campbell et al., 2004). In human or animal stress studies, lateralization is in general poorly addressed, even though clear effects have been found in rodents, and in the hippocampus of chronically stressed pigs, where a significant correlation was found between salivary cortisol values, apoptosis and dentate gyrus volume only in the left, but not right hippocampus, suggesting that lateralization effects are not limited to humans (Van der Beek et al., 2004; Carlson and Glick, 1989, 1991; Delrue et al., 1994; Tabibnia et al., 1999).

There is a strong relationship in humans between the duration of the depression and the extent of atrophy (Sheline et al., 1996, 1999). In this study, depressed patients who received electroconvulsive therapy were not excluded initially, although animal experiments...
suggest that seizures can produce neuronal loss and gliosis in the hippocampus, but also provide a major stimulus for dentate neurogenesis (Madsen et al., 2000). On the other hand, posthoc exclusion of these patients did not change the results. A recent meta-analysis on hippocampal volume in depressive patients further confirmed a reduced volume in this disorder and attributed part of the discrepancy between literature findings to inclusion of the amygdala and to clinical variables of the populations, like duration of illness and presence of abuse (Campbell et al., 2004). In general, it cannot be excluded that the reported differences are due to pharmacological treatment of depression rather than to hypercortisolaemia, or that a small hippocampal volume predisposes for depression or PTSD (Sapolsky, 2002; Wignall et al., 2004). One MRI study found significantly shortened T1 relaxation times for the hippocampus in depressed patients, especially in the elderly ones, indicating differences in the water content of the hippocampus (Krishnan et al., 1991). As we could not find any significant histological damage in the hippocampus of depressed patients, changes in water content at least provide an alternative explanation for hippocampal atrophy (Lucassen et al., 2001a; Müller et al., 2001).

A second possible explanation could be modulation of the turnover rate of neurons in the adult dentate gyrus. Both apoptosis and neurogenesis continue to occur in this subregion into adulthood in mammals, including humans (Eriksson et al., 1998). As glucocorticoids and stress suppress neurogenesis in this area, prolonged HPA-axis activation may, over time and assuming unaltered rates of apoptosis, cause a reduction of the total hippocampal volume due to a disturbed balance in the cellular turnover in this area (Kempermann and Kronenberg, 1999). Chronic unpredictable stress in rats indeed differentially modulated both cell death and cell birth (Heine et al., 2004a,b). Also, a study in psychologically stressed tree shrews, considered to be a good model for HPA alterations in depression, showed reduced hippocampal volume, associated with reduced numbers of BrdU-positive newborn cells, which, interestingly, normalized following antidepressant treatment (Czéh et al., 2001; Fuchs et al., 2001; Lucassen et al., 2001b). Similarly, the chronic stress induced subregion-specific changes in hippocampal apoptosis normalized after antidepressant treatment (Lucassen et al., 2004), while, interestingly, normalization also occurred in the associated cortical areas, indicating a general anti-apoptotic mode of antidepressant action not restricted to the hippocampus alone. In addition, it suggests that the low numbers of apoptotic cells found previously in hippocampal tissue of depressed patients, may in fact even have been an underestimation, as almost all patients were or had been on antidepressant treatment prior to their death (Lucassen et al., 2001a).

In Vietnam combat veterans diagnosed with PTSD an atrophy of the hippocampus was reported in a number of studies. These patients experience flashbacks, nightmares and suffer from sleep problems, emotional numbness or emotional outbursts, anhedonia, inappropriate startle reflexes and problems with memory and concentration (Sapolsky, 1996; Bremner, 1999). Also, deficits in their short-term verbal memory were associated with a smaller right-side hippocampal volume (Bremner et al., 1995, 1997; Bremner, 1999). Victims of childhood abuse may on the other hand have a smaller left hippocampus. It is unlikely, however, that an excess of glucocorticoids caused the hippocampal atrophy in these patients. Although Sapolsky (1996) proposes that these changes would be due to irreversible neuron loss, or altered turnover (Sapolsky, 2001), neuropathological examination of the hippocampus has not been performed in any of these patients.
Moreover, since prospective studies are lacking, it cannot be excluded with certainty that the relationship between the various disorders and smaller hippocampi is the opposite of the one proposed in literature (Sapolsky, 1996, 2001), i.e., that smaller hippocampi are a risk factor for depression and PTSD (Sapolsky, 2002; Wignall et al., 2004), or even for Cushing’s disease. Strong evidence has recently been obtained for this alternative explanation by a study of 40 pairs of identical twins, one of whom went to the Vietnam war and experienced combat, while the other stayed at home. Of those who experienced combat, 43% developed PTSD and had smaller hippocampi. However, their stay-at-home twin brothers also had small hippocampi. A small hippocampus thus seems to be present already prior to the stressful experience and may confer an increased vulnerability to PTSD (Gilbertson et al., 2002).

Furthermore, it is questionable whether hypercortisolism is indeed responsible for the hippocampal atrophy, since combat-related PTSD patients do have smaller hippocampi, but no hypercortisolism is found. On the contrary, this disorder is associated with decreased HPA-axis activity and steroid feedback supersensitivity, that often lasts for decades after the initial trauma (Yehuda et al., 1995a). It has been presumed that early on in the process the HPA-axis may have been strongly activated. This is based on the observation that soldiers who had undergone random bombardments in the Korean war had markedly increased levels of cortisol, with the highest levels of cortisol in soldiers who had been in the greatest danger (Bremner, 1999). It has therefore been hypothesized that high levels of cortisol at the time of the stressor would result in damage to the hippocampal neurons, that may persist for many years after the original trauma and that could lead to reductions in hippocampal volume and subsequent differences in feedback or stress responsivity (Bremner, 1999). However, those victims of rape or motor vehicle accidents who later developed PTSD appeared to have, within a few hours after the traumatic event, lower cortisol levels than victims who had no subsequent psychiatric disorder or those who developed major depression. Yet, pituitary and adrenal hyperactivity to exogenous CRH and ACTH has been demonstrated in these patients. An increased sensitivity or upregulation of glucocorticoid receptors in PTSD and a pre-existing smaller hippocampal volume thus seems, at present, the best explanation for all the data (Yehuda, 2001; Rasmusson et al., 2001; Sapolsky, 2002).

In animal studies on subordinate, wild born velvet monkeys that died spontaneously after prolonged severe social stress in captivity, adrenal hypertrophy was found postmortem. The coincident hippocampal degeneration in these animals was interpreted as support for the glucocorticoid cascade hypothesis. The morphological alterations and neuron loss in these animals were most pronounced in Ammon’s horn pyramidal neurons (Uno et al., 1989) brain regions particularly sensitive to physical perturbations or postmortem compression trauma (Van den Pol and Gallyas, 1990; Cammermeyer, 1978, 1979). However, a later controlled experiment with male tree shrews (Tupaia belangeri) that had been exposed to subordination stress for 28 days, which resulted in continuously elevated urinary cortisol levels, did not support this observation. The number of pyramidal neurons in hippocampal field CA1 and CA3 as determined by the optical fractionator technique appeared not to be significantly altered in comparison to unstressed controls (Vollmann-Honsdorf et al., 1997). Also, no increased apoptotic cell death could be demonstrated in these areas in the same model (Lucassen et al., 2001b; Fuchs et al., 2001).
Together, this pleads against the relevance of the proposed glucocorticoid cascade hypothesis in sustained stress.

1.13. Other observations that do not support the possible importance of the glucocorticoid cascade

There are, in addition, various observations that do not support the possible importance of the glucocorticoid cascade for the pathogenesis of AD.

First, hypercortisolism in AD is mild. The literature on this topic is even ambiguous. Although some papers reported baseline levels of cortisol to be elevated in plasma and urine of AD patients (Davis et al., 1986; Maeda et al., 1991; Umegaki et al., 2000) others failed to do so (Ferrier et al., 1988). One study even indicated that, as a group, Alzheimer patients have a mildly increased HPA-axis activity, but the increased baseline cortisol levels were not stable longitudinally and did not increase when followed up at later time-points, which is not consistent with the glucocorticoid cascade hypothesis (Swanwick et al., 1998). Another study reported that in relatively early stages of AD high plasma cortisol levels led to rapid cognitive decline (Umegaki et al., 2000), whereas others were unable to find increased salivary cortisol levels in mild cognitive impairment, a condition considered to be a risk factor for AD patients (Wolf and Kirschbaum, 2002). Postmortem CSF cortisol levels in AD patients were indeed 83% higher than in controls. In presenile patients these levels were even five times higher than in controls. However, due to the increasing cortisol levels in the course of normal aging, significant differences in CSF cortisol levels were no longer found between senile AD patients and (aged) controls (Swaab et al., 1994), which is in agreement with the similar blood levels we found in these groups (Swaab, 2003). Increased basal plasma and CSF cortisol levels thus do not seem to be necessary for the development of the pathogenesis of AD (Swaab et al., 1994). On the other hand, lumbar puncture CSF levels of cortisol were found to be increased in AD in an APOE genotype-dependent way. APOE-4 went together with higher cortisol levels (Peskind et al., 2001). APOE-4 is indeed a major risk factor for AD. However, this observation may be explained by the stronger AD changes in the hippocampus of APOE-4 positive subjects, which would cause a stronger disinhibition of the HPA-axis.

The cortisol neurotoxicity hypothesis furthermore does not explain why in some AD patients, despite extensive neuropathology, ventricular cortisol CSF levels are not elevated, and why the dexamethasone suppression test is only disturbed in 50% of the AD patients (for references see Swaab et al., 1994). Another argument against the importance of the cascade hypothesis is that in early stages of AD basal plasma levels of ACTH, cortisol and the dexamethasone suppression test were all normal, at least in some studies (Francescheschi et al., 1991, but see Umegaki et al., 2000). Furthermore, we did not find a difference in basal cortisol and DHEAS between controls and AD patients (Swaab, 2003). Others reported that hippocampal perfusion diminishment, as measured by SPECT, correlated with decreased DHEAS levels rather than with increased cortisol (Murialdo et al., 2000).

However, one of the strongest arguments against the causal involvement of the glucocorticoid cascade in AD is that no AD or other neuropathological changes are present in the hippocampus of depressed patients, nor in patients treated with glucocorticoids (see below), despite a much stronger activation of the CRH neurons in depression than in AD.
If cortisol neurotoxicity would indeed be a major component in the pathogenesis of AD, no explanation can be given for these and other disorders, in which at least a similar extent of hypercortisolism is found, but where no accompanying AD changes occur in the hippocampus, such as in patients suffering from Cushing’s disease (Trethowan and Cobb, 1952), depression, multi-infarct dementia (Maeda et al., 1991), multiple sclerosis (Purba et al., 1995; Erkut et al., 1995) or in patients that received (synthetic) glucocorticoids for a variety of reasons (Lucassen et al., 2001a; Müller et al., 2001; O’Brien et al., 2001a,b). Because of the presumed neurotoxicity of cortisol in depression, we studied the hippocampus of 15 patients that were well established to be severely depressed, 9 glucocorticoid treated patients and 16 controls. In Haematoxilin-Eosin, silver (Bodian) staining, Nissl (thionine), hyperphosphorylated-tau (Alz-50), B-50, synaptophysin stained sections (Figs. 8 and 9), and following in situ end-labeling for DNA fragmentation, immunocytochemistry for the inducible form of heat shock protein 70, and nuclear transcription factor kappa-B, no indications for AD changes or obvious massive cell loss could be observed. The absence of any major pyramidal cell loss and the very rare occurrence of apoptosis (Fig. 10), notably absent from areas at risk for glucocorticoid damage like CA3, indicates that apoptosis has probably only contributed to a very minor extent to the volume changes in these conditions and that other mechanisms must have been involved (Müller et al., 2001; Lucassen et al., 2001a). Another independent postmortem study on depressed patients also concluded that the liability for some patients to develop cognitive impairment during a depressive episode was not related to an increase in AD or vascular neuropathology (O’Brien et al., 2001a). This agrees with the general clinical experience with depressive or Cushing’s patients, in which antidepressant treatment, psychotherapy or removal of the tumor can normalize and relieve the depressive symptoms, HPA alterations, and even the hippocampal atrophy (Starkman et al., 1999; Rowe et al., 1997). This would be difficult to imagine should massive cell loss indeed have occurred.

Consistent with this, the CA3 atrophy in (Magarinos and McEwen, 1995) rat and tree shrew hippocampus after chronic stress or glucocorticoid excess disappeared once the treatment was stopped or antidepressant treatment commenced (e.g., Czéh et al., 2001). In addition to the human studies, various recent anatomical reports on the hippocampus of stress or steroid exposed (aged) rodent or (aged) monkeys, utilized dissector and stereological tools (West, 1999) for an accurate quantification of hippocampal cell numbers and volume, and failed to find massive structural loss in the main pyramidal and granular layers (Heine et al., 2004a; Leverenz et al., 1999; Sousa et al., 1998a,b, 1999; Rapp et al., 1999; Rapp and Gallagher, 1996; Vollmann-Honsdorf et al., 1997; Rasmussen et al., 1996). Since in most older studies, shrinkage-sensitive density measures were used to assess structural changes, this may have caused a bias when counting cell numbers, and also methodological differences may therefore have contributed to the previous results in chronically stressed rats (Swaab and Uylings, 1987; Sapolsky et al., 1986; Stockmeier et al., 2004).

Furthermore, other important structural components of the dentate gyrus that are affected by stress are the ongoing neurogenesis and apoptosis in this region (Heine et al., 2004b). The mature neuronal population of the DG provides strong projections to the CA3 region. Stress-related modulation of this heterogeneous population of old and new neurons in the DG provides interesting opportunities for functional adaptation of the hippocampus. As “strategic gatekeepers” (Kempermann, 2002; Kempermann and Kronenberg, 1999),
stress effects on DG neurons are likely to be reflected by changes in, e.g., synaptic transmission at the CA3 synapses or dendrites. Indeed, alterations in DG turnover occur prior to stress effects on the CA3 dendritic trees, that need more time to develop and are longer lasting (Heine et al., 2004b; Pham et al., 2003).

Although the exact mechanisms responsible for this hippocampal volume loss have not yet been identified, both dendritic atrophy and reductions in neurogenesis have been
Fig. 9. Representative photomicrographs showing the immunohistochemical staining with an antibody against synaptophysin (A, C and E) and the neuronal phosphoprotein B-50 (B, D and F) in the hippocampus of a depressed patient (A and B), a steroid-treated patient (C and D) and a control subject (E and F). Synaptophysin-like immunoreactivity in the CA3 pyramidal area (A, C and E) reveals the typical, strong, punctate staining of the neuropil, particularly in the stratum lucidum of the CA3 pyramidal area, where the mossy fibers form giant en passant synapses, the characteristic mossy terminals, on the proximal dendrites of the CA3 pyramidal neurons. Immunohistochemical staining for the neuronal phosphoprotein B-50 shows the characteristic strong B-50 immunoreactivity in the dentate gyrus' molecular layer (ml), the region of the apical dendrites of the granule cell (gc). No marked difference can be observed between the immunohistochemical staining patterns of depressed patients (A and B), steroid-treated patients (C and D) and control subjects (E and F) in the hippocampal subarea CA3 and the molecular layer of the dentate gyrus, both areas predicted to be at risk for glucocorticoid overexposure (from Müller et al., 2001, Fig. 2, with permission).
implicated in the selective volume reduction in stress-related neuropsychiatric illnesses such as major depressive disorder, but also in experimental animals (Manji et al., 2003; Ohl et al., 2000; Sheline, 2000; Sheline et al., 2003). The accumulation of changes in neurogenesis over time might affect hippocampal volume, assuming other structural parameters such as apoptosis remain unaltered. Stress-induced decrease of the hippocampal volume and the parallel reduction in cytogenesis could indeed be prevented...
by antidepressant treatment (Czéh et al., 2001; Van der Hart et al., 2002). Furthermore, hippocampal neurogenesis was shown to be needed for certain antidepressant drugs to become effective (Santarelli et al., 2003). In addition, these stress-induced changes are reversible after an appropriate period of recovery, indicating transient and adaptive effects of stress. However, hippocampal neurogenesis adds relatively few neurons per day (Cameron and McKay, 2001; Heine et al., 2004b) and only to the granule cell layer of the dentate gyrus, where similar numbers of neurons die (Kempermann and Kronenberg, 2003). Conversely, reducing the number of newborn cells by means of radiation, is not paralleled by the occurrence of depressive symptoms (Henn and Vollmayr, 2004). Even though alterations in dentate turnover may, with time, have considerable structural and functional consequences for the hippocampus, it is unlikely that disturbances in hippocampal neurogenesis alone will fully explain a disorder as complex as major depression. Other explanations for the prominent hippocampal atrophy in depression are that the volume loss is due to alterations in the dendritic and synaptic components or in the glia cells within the hippocampal network (Fuchs et al., 2004; Rajkowska, 2000). Furthermore, a more mechanistic explanation of hippocampal volume changes could be a shift in fluid balance between the ventricles and brain tissue as supported by various clinical studies reporting on enlarged ventricles, parallel to lower volumes of different brain structures in patients with affective disorder (reviewed by Manji and Duman, 2001; Manji et al., 2003). Most of these changes are transient, reversible and adaptive, rather than neurotoxic.

In conclusion, the HPA-axis is only moderately activated in AD, possibly resulting from, rather than causing, the hippocampal neurodegeneration in this disorder. There are no convincing arguments to assume a causal and primary role for cortisol in the pathogenesis of AD. CRH and cortisol might, however, be causally involved in the development of depression. Although there is no evidence for any major damage in the human hippocampus in depression or following glucocorticoid treatment, subtle changes that have gone unnoticed with the present techniques can, of course, at present not be excluded.

2. Conclusions

The final common pathway of the stress axis is the HPA-axis. During aging the HPA-axis is gradually activated. In AD a moderate activation and in depression a strong activation of the stress axis occurs. This activation is driven by the CRH-containing neurons, that start to coexpress vasopressin when chronically activated, which potentiates CRH actions.

CRH and cortisol act centrally and are implicated in depression. In this disorder the vasopressin neurons of the SON are also activated, which is related to an enhanced suicide risk. Also, changes in the vasopressin neurons of the SCN, the biological clock, occur, that cause sleep disturbance and contribute to CRH activation.

In depression, the HPA-axis is activated while the activity of the pituitary HPG-axis is diminished. This explains the lower plasma levels of estrogens and the higher adrenal androgen levels in depressed women and the lower testosterone levels in depressed men.
Sex hormones act on the HPA-axis, and changes in their levels are a risk for depression. Some observations further indicate that hormone replacement therapy is beneficial in mood disorders.

In depressed subjects or in patients treated with synthetic corticosteroids the hippocampus is intact and does not show any indication of neuropathological alterations or major structural damage in postmortem material. We conclude that in the human brain there is no conclusive evidence that corticosteroids would be neurotoxic for the hippocampus.

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