General discussion

Study approaches
The central question of this thesis is whether major depressive disorder (MDD) and depression in Alzheimer’s disease (AD) share their pathophysiology. Despite the fact that the pathophysiology of depression is not known, there are a number of neurobiological hypotheses, one of which concerns the hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis. In the present thesis we have addressed the central question by investigating the HPA-axis hypothesis in both MDD and depression in AD. The findings in this thesis aim to contribute to the development of new therapeutic strategies for MDD and depression in AD, targeting the release and effects of hypothalamic neuropeptides, especially CRH and vasopressin.

There are several possible approaches to study brain changes in depression. Two major approaches are 1) studies in animal models of depression and 2) post mortem brain studies in humans. We will first discuss the advantages and disadvantages of post mortem research, especially in relation to animal research.

Disadvantages of post mortem brain research
1. Post mortem research does not allow the study of how tissue functions. At best we can ‘capture’ the state of the brain at death: the extent to which this succeeds depends on several known and unknown processes that begin after death and that may affect the integrity of the neurons (Swaab, 2003). To limit the damage of such destructive processes the human brain must be fixed as soon as possible after death (Swaab, 2003). In the group of the AD patients, the post mortem delay, i.e. the time between the moment of death and fixation of the brain material, was, in general, exceptionally short, often no longer than a few hours. The reason for this is that the patients included in the cohort of prospectively followed AD patients resided in a nursing home in Amsterdam, not far from the VUmc where the autopsies take place. Moreover, all the required permissions and information about the procedures that had to be followed were taken care of long in advance by the Netherlands Brain Bank (NBB). In the group of depressed subjects and controls the post mortem de-
lay was much longer, but this did not make a difference to our experiments. But it is not only after death that changes may occur. The process and especially the stress of dying may have a profound influence on the brain, in particular on the HPA-axis. The changes that occur during a depressive state may thus be dølged by the changes that occur as a result of the agony of dying (Swaab, 2003). However, even though the HPA-axis is activated by the stress of dying, its activity stage during life is still reflected in the post mortem period (Erkut et al., 2004). Moreover, the control subjects had also undergone the process of dying, so that the differences we report between patients and controls are not due to this process of dying itself. However, differences that existed before dying may no longer be present after death, because brain changes occur during the dying process. Animal studies, too, face the effects of the process of dying, although in animals it can be kept under control and limited.

2. Scarcity. Post mortem human brain material is scarce, not only in the Netherlands, but throughout the world. The collection of human hypothalami obtained via the NBB currently amounts to approximately 2400 (Swaab, 2003). In spite of this high number, the groups of psychiatric patients, generally, will remain small: about 10-20 subjects per group. In animal research this problem of scarcity can usually be circumvented.

3. Brain material, when collected over a long period, is handled according to the procedures and techniques of that time. The material we used was embedded in paraffin (Swaab, 2003). Quantitative immunocytochemistry and in situ hybridization are very well possible on this material. However, new techniques such as micro-array and qPCR do not work well on paraffin-embedded material and require frozen brain material. Animal research can be adapted immediately to new techniques.

4. Human life is not ‘controlled’ (Swaab, 2003). People who donate their brains to the NBB go on with their lives. They individually eat, drink, and take medication, and all these factors may influence brain areas or circuits that are subject of research. For instance, the use of corticosteroids will influence HPA-axis functioning. In animal research all this can be controlled.

5. It takes years to build a collection of brain material (Swaab, 2003), and with the psychiatric classification changing and a new DSM appearing every 15 years, the diagnoses made during life as mentioned in the medical record of the patients are in some cases not in accordance with the present-day criteria. Therefore, we have added in our studies on depressed subjects a post mortem evaluation of the given data with respect to present day DSM-IV criteria. In animal research none of this applies.

6. Patients were considered `depressed’ when they had suffered from a depression
or depressive episode at some point in their lives. When people with a psychiatric disorder decide to donate their brains to the NBB, there is usually a long period of time between the last acute episode and the time of death when their brain material is acquired. The process of aging and the accompanying detrimental effect on the brain (Swaab, 2003) may hamper the possibility of getting the correct impression of the biological status of the brain during an acute psychiatric status. In addition, depression tends to run a chronic course and patients usually recover. Therefore, at the moment of death, some ‘depressed’ donors were probably not actually depressed. However, in our study, most of the patients were depressed at the time of death. In addition, the course of depression of the subjects in the collection of the NBB was usually severe, as can be seen in Chapter 2, where 6 out of 9 depressed patients could be diagnosed as melancholic type depression, and 3 out of the 9 depressed subjects committed suicide. In animal research a delay between the presumed mood state and death does not occur.

7. Bar a few exceptions, the information on the subjects has not been gathered prospectively (Swaab, 2003). Clinical information about NBB brain donors often has to be completed after autopsy. In four of the studies described in this thesis, however, prospectively assembled information was available: the AD patients studied were part of a prospectively followed cohort of demented patients, living in eight nursing homes in Amsterdam. The patients were studied at six-month intervals, focusing on diagnostics for dementia/AD and on depression scales. For a detailed discussion of the composition of the group of AD patients, see Hoogendijk, 1998.

Advantages of post mortem brain research

At the same time, human brain material has two important advantages:

1. The material is of humans with the genuine disease and not from a “model” for the disease. This is a clear advantage compared to animal research, which is usually performed on rat and mice models for features of the disease. This is especially important as far as studies of psychiatric disorders are concerned. For example, it is very difficult or even impossible to determine most features of a depressive episode in animals. These features are: (1) depressed mood most of the day, nearly every day, as indicated by either subjective report (e.g., feels sad or empty) or observation made by others (e.g., appears tearful), (2) markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation made by others), (3) significant weight loss when not dieting or weight gain (e.g., a change of more than 5% of body weight in a month), or a decrease or increase in appetite nearly every day, (4) Insomnia or hypersomnia every day, (5) Psycho-
motor agitation every day (observable by others, not merely subjective feelings of restlessness or being slowed down), (6) fatigue or loss of energy nearly every day, (7) feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick), (8) diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others), (9) recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide (American Psychiatric Association, 1994). These symptoms require an operationalization to be used in animal studies, if that is at all possible.

In addition, to diagnose a major depressive disorder, and not just a depressive episode, one has to meet the criterion of 1) causing clinically significant distress or impairment in social, occupational, or other important areas of functioning and 2) symptoms not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition (e.g., hypothyroidism), and 3) the symptoms are not better accounted for by bereavement, i.e., after the loss of a loved one the symptoms persist for longer than 2 months or are characterized by marked functional impairment, morbid preoccupation with worthlessness, suicidal ideation, psychotic symptoms, or psychomotor retardation (American Psychiatric Association, 1994).

In conclusion, it is not an easy task to establish whether an animal fulfills the criteria of a depressive episode, let alone a MDD. Especially the features that may distinguish a depressive episode from physical illness are hard or impossible to determine in animals, such as feelings of worthlessness or excessive or inappropriate guilt and recurrent thoughts of death, recurrent suicidal ideation, or a plan for committing suicide. In addition, these features are of great clinical importance, since they are related to mortality in depression (Nemeroff et al., 2001; Ebmeier et al., 2006). Animal models only reflect symptoms. They do not present the human phenotype of depressive illness. One could even doubt whether ‘depressive episodes’ in a clinically significant sense occur in rodents at all. In any case it will be evident that animal experiments in psychiatric disorders are more complicated than, e.g., in studying wound-healing or diabetes. Studies of psychiatric disorders in monkeys, whose brains and behaviour resemble ours more than those of mice and rats, are rare (Barr et al., 2004). The paradox of, particularly, monkey research is that the more it succeeds in convincing that monkeys resemble us, the more problematic it may be from an ethical point of view to perform the experiments.

2. Cellular and molecular studies of the human brain in living subjects are only possible to a very limited degree via neuroimaging. While samples may be readily
taken from many types of tissue, for the brain, even if the skull can be bypassed, this is not feasible, apart from the fact that no crucial information can be obtained that way. Psychiatric research in the field of brain imaging in humans has taken a tremendous flight, but cannot yet give functional information on the small hypothalamic nuclei, and post mortem human material thus remains essential for depression research.

The two advantages, i.e. that the material is human and that it can be studied at the microscopical and chemical level, are not simply outnumbered by the disadvantages. For every research question the appropriate approach, be it animal research or post mortem research, will have to be chosen. Still, it is the ongoing challenge for post mortem research to minimize the disadvantageous factors mentioned above and to make use of the advantages.

Chapter 2: Vasopressin in depression

Using in situ hybridization we observed that in depressed subjects the amount of AVP mRNA is increased in the SON (Table 1), which points to the SON as the main source of the observed increased plasma levels of AVP in depression (Van Londen et al., 1997). Also, whereas until now the PVN has been the focus of hypothalamic HPA-axis research (Dinan et al., 2005), the results draw attention to the SON as a factor in HPA-axis activation in depression. In addition, our data indicate that the increase in AVP mRNA is even more pronounced in melancholic-type depression, which is in accordance with earlier studies that found elevated AVP plasma levels to be particularly related to melancholic-type depression. The technique of in situ hybridization implies that the anatomical structure of the brain tissue remains intact. This is of importance in the hypothalamus, since we measure the AVP mRNA in very small nuclei for whose identification the preservation of the anatomical structure is of great importance. However, in situ hybridization has the disadvantage that it can only measure one type of mRNA at the time. Still, it would be interesting to determine not only the amount of AVP mRNA but also the amount of OXT mRNA (see Chapter three), CRH mRNA, messengers of receptors and input of aminergic systems in the same experiment. Such simultaneous measurements can be performed by qPCR. This would not only be quicker, but it would also provide information about the pathophysiological relationship between the expression of the different types of mRNAs. Unlike some other medical disorders, in which an anomalous gene is expressed (like in Duchenne (Chakkalakal et al., 2005)) or a gene product is absent (as in Prader-Willi syndrome, deletion on chromosome 15 (Nowaczyk et al., 2004)), in psychiatric disorders the causes are probably mostly multifunctional
and the gene expression differences more subtle and best studied from the perspective of the interplay and changed profile of several genes in different brain areas. For future experiments it will be very valuable to use a combination of laser dissection microscopy with qPCR on frozen and cryostat-sectioned material to be able to say more about the profile, and possibly interplay, of the expression of genes supposed to be involved in HPA-axis regulation in affective disorders. The collection of brain material from depressed donors is currently, however, the rate-limiting factor.

Table 1. Changes in the paraventricular and supraoptic nucleus in major depressive disorder and depression in Alzheimer’s disease

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AD, Alzheimer’s disease; CRH, corticotropin-releasing hormone; PVN, paraventricular nucleus; AVP, arginine vasopressin; OXT, oxytocin; MDD, major depressive disorder; nd, not determined; PVN, paraventricular nucleus; SON, supraoptic nucleus. ↑↑: increased number/amount; ↑↑: unchanged number/amount. ↑↑ positively correlated with Cornell scale for depression in dementia. ° in melancholic-type MDD. °Melancholic-type increased compared to non-melancholic-type depression.
The possibility mentioned in Chapter 2, that hypothalamic AVP expression is related to suicidal behaviour in depression (Inder et al., 1997), is supported by a recent study by Merali et al. that determined the alterations in neuropeptides (among which CRH, AVP) in stressor-sensitive brain regions of subjects who committed suicide compared to individuals who had died of other causes than suicide. In subjects who had died from suicide, elevated levels of AVP-immunoreactivity were found within the PVN, though not in the median eminence (Merali et al., 2006). Regrettably, Merali et al. did not include the SON in their study, although this is a major source for circulating AVP (Ishunina et al., 2002). The results in Chapter 2 emphasize the importance for future research of including the SON in studies on possible factors involved in suicide.

MDD is known to be a risk factor for cardiovascular disease, while the pathophysiological relationship is unknown (O’Connor et al., 2000). Interestingly, Weissman et al. found that offspring of depressed parents already reported more medical illness, at an average age of 35 years, than the offspring of non-depressed parents, particularly cardiovascular problems, which were more than five times as likely, while neuromuscular disorders were only twice as likely (Weissman et al., 2006). Elevated levels of AVP are related to hypertension, as was found by Zhang et al. in a population-based sample (Zhang et al., 1999), and AVP antagonists are proposed to be beneficial in the treatment of hypertension and congestive heart failure (Mayinger et al., 1999). It may be hypothesized that an increased hypothalamic vasopressinergic drive is a common pathogenic mechanism of both MDD and cardiovascular disease. Further research is required concerning the role of AVP in depression and riskful somatic comorbidity. Our data indicate the importance of including the SON in future post mortem and animal research.

Chapter 3: Oxytocin in depression
In our study, it turned out that melancholic-type depressed patients had significantly higher OXT mRNA in the PVN than non-melancholic-type depressed patients (Table 1). We also observed a trend towards higher OXT mRNA in the SON in melancholic-type depressed patients compared to non-melancholic patients (p=0.070; these latter data are not shown in Chapter 3 and have not earlier been published). In addition, there was a trend towards increased OXT mRNA in the SON of melancholic-type patients compared to control subjects. In the previous Chapter we observed that the AVP mRNA level was significantly increased in the SON, with a significant increase in both PVN and SON in melancholic depression compared to controls. The increases of AVP and OXT in the PVN and SON seem to be related, since 1) there was a correlation
between the amount of OXT mRNA in the PVN and SON both in depressed subjects \(( \rho = 0.849, \ p = 0.004 )\) and in controls \(( \rho = 0.733, \ p = 0.025 )\). Data not shown in Chapter 3), 2) the amount of AVP mRNA in the PVN and SON was correlated both in depressed subjects \(( \rho = 0.8, \ p = 0.01 )\) and in controls \(( \rho = 0.8, \ p = 0.01; \) data not shown in Chapter 2), and 3) a correlation existed between the amount of AVP mRNA and the amount of OXT mRNA both in the PVN and in the SON in depressed subjects (Meynen et al., 2007a). Since these correlations exist, a differential expression of OXT and AVP in the PVN and SON does not seem to take place. Rather, in melancholic-type depression both AVP and OXT expression are increased in both nuclei, but due to the small groups we did not find a significant difference in either of the nuclei.

Interestingly, using immunocytochemistry, Sivukhina et al. showed that corticosteroid-binding protein (CBP) is co-localized with AVP and even more with OXT in the human hypothalamus, a finding similar to the situation in rat (Sivukhina et al., 2006). CBP may regulate corticosteroid actions by altering local hormonal concentrations or through interactions with intracellular adenylate-cyclase/cAMP second messenger generating pathways (Sivukhina et al., 2006). The finding of Sivukhina et al. suggests that OXT is as much a factor in the regulation of the stress axis as is AVP. This could support the hypothesis of Legros, that both AVP and OXT regulate the HPA-axis as “ying-yang” hormones, where while AVP stimulates HPA-axis activity, OXT attenuates its activity (Legros, 2001).

Recently, Emiliano et al. investigated the distribution and overlap of OXT-labeled cells and serotonin transporter (5-HTT) immunoreactive fibers in the macaque PVN and SON (Emiliano et al., 2006). They found that in these nuclei the distribution of 5-HTT-labeled fibers follows the distribution of OXT-labeled cells. Their findings indicate that the therapeutic effects of SSRIs may, in part, be mediated through components of OXT in the PVN and SON. Although the macaque hypothalamus anatomically resembles the human hypothalamus more than the rat hypothalamus does (Emiliano et al., 2006; Swaab, 2003; Koutcherov et al., 2000), it would still be important to confirm these experiments in post mortem human tissue, including a group of depressed subjects, preferably with and without SSRI treatment. This may contribute to a further understanding of how the serotonin-hypothesis of depression may be related to the HPA-axis-hypothesis of depression.

Neumann et al. studied the role of intra-SON and intra-PVN OXT in the regulation of local AVP release and into the blood in male rats after forced swimming. They demonstrated a receptor-mediated effect of OXT within the SON and PVN on local and neurohypophyseal AVP release. They found that,
while exerting an inhibitory effect on hypothalamic-pituitary-adrenal axis activity under basal conditions, hypothalamic OXT is essential for an adequate acute ACTH response (Neumann et al., 2006).

**Chapter 4: Cortisol in AD**

In this study we confirmed the earlier finding of increased CSF cortisol in AD (Swaab et al., 1994; Erkut et al., 2004). We did, however, not find a difference between depressed and non-depressed AD subjects, nor did we find a correlation between the CSF level of cortisol and the Cornell scale for depression. For this study we used the subdivision of the AD patients that was also used in earlier studies (Hoogendijk et al., 1999a; Hoogendijk et al., 1999b; Liu et al., 2000). AD patients were divided into three groups: 1) AD patients without affective disorder during their entire life (‘AD patients without depression’), 2) AD patients who had suffered from depression during at least the last three months of their lives, and 3) patients that did not fulfil the criteria of either group. AD patients in this third group were called ‘transiently depressed AD patients’. The first point that may be raised concerning this division is that the requirement of a depressive state of three months is arbitrary. The diagnosis of MDD itself implies a criterion of time (two weeks) and it does not seem necessary to introduce an additional time criterion. It is, for instance, unlikely that there would be a pathophysiologically relevant distinction between the states of patients who suffered from a 2- or 3-month depression, respectively. Second, the group of transiently depressed patients consists of a heterogenous group of patients. It included, for instance, AD patients who suffered from ‘dysthymia’ during the course of AD and patients who suffered from depression during their lives, but not during the course of AD. We could, therefore, of course, have excluded these patients from the study. Another approach is, however, to use the advantage of the prospective nature of the study, and to use the last measurement before death as the best indication of the pathophysiological state at the time of death. In the following studies (Chapters 5, 6 and 7) we have chosen this approach. This means that in Chapters 5 and 6 the group of AD patients is divided into two subgroups instead of three: those with and those without a depression, diagnosed at the last measurement before death. This is also more in line with the use of the last Cornell as was done in the present experiment (Chapter 4) and in previous studies (Liu et al., 2000; Hoogendijk et al., 1999a; Hoogendijk et al., 1999b). It should be noted, that reanalysis of the data of Chapter 4 using this criterion of two groups did not result in a significant difference in CSF cortisol level between depressed patients and non-depressed AD patients (p=0.3).
Cortisol is not the only glucocorticoid in the CSF. A recent study investigated the role of corticosterone in the human HPA-axis (Raubenheimer et al., 2006). It was found that corticosterone makes up almost 40% of the total active glucocorticoids, i.e. both cortisol and corticosterone, in the CSF. However, the fact that significant effects on HPA-axis suppression were only seen with supraphysiological levels of corticosterone suggests that corticosterone is not a major factor in HPA-axis feedback in the non-stress-induced HPA-axis activity in this study, in which the effect of cortisol predominated (Raubenheimer et al., 2006).

Since cortisol levels are not related to the presence of depression in AD, the question arises about the meaning of the elevated cortisol levels as they have now been observed several times in AD in both post mortem (Swaab et al., 1994; Hoogendijk et al., 2006) and lumbar (Peskind et al., 2001) CSF, and how they are related to the disease process, if not via depression. The hippocampus plays a central role in the inhibition of HPA-axis activity (Jacobson et al., 1991). It may be hypothesized that damage to the hippocampus as it occurs in AD leads to disinhibition of the HPA-axis, and, more specifically, to an increase in cortisol levels (Csernansky et al., 2006) (see also the discussion of Chapter 7). In addition, Peskind et al. found lumbar CSF cortisol levels to be related to increased frequency of the APOE4 allele and decreased frequency of the APOE2 allele in AD subjects relative to control subjects (Peskind et al., 2001). So far no relationship with depression has been made. The fact that CSF cortisol levels are not further increased in depressed AD patients compared to non-depressed AD patients might also be due to a ‘ceiling’ effect, where in depression in AD the HPA-axis would already be maximally activated. It is possible that the HPA-axis, at the level of the hypothalamus, pituitary or adrenal gland, is already maximally active in AD patients undergoing the process of dying, since this process leads to a twenty-fold increase of the CSF cortisol level (Erkut et al., 2004; Swaab et al., 1994). The increase in post mortem CSF cortisol levels is in accordance with the marked increase in cortisol level in critically ill patients that has been observed in vivo (Reincke et al., 1993).

Chapter 5: CRH in AD

A robust finding in relation to the hypothesis of increased HPA-axis activity in the pathophysiology of depression is the fourfold increase of the number of CRH-expressing neurons in the PVN in MDD (Raadsheer et al., 1994a). A further indication for activation of these neurons in depression was the observation that the number of CRH neurons colocalizing AVP was increased three times in depressed subjects (Raadsheer et al., 1994a). A third point is the increase of
CRH mRNA in MDD (Raadsheer et al., 1995). Given our research question of the pathophysiology of depression in AD versus MDD it was thus of great importance to determine whether depression in AD is also accompanied by an increase in CRH expressing neurons. Meanwhile, since it was already known that the number of CRH expressing neurons does not increase in AD (Raadsheer et al., 1994b), it was likely that when an increase would exist in depressed AD patients it would be moderate and not as robust as in MDD.

We did indeed find a 40% increase in CRH neurons in depressed AD patients compared to controls, but this increase was not significant (Meynen et al., 2007b). We did observe, however, a significant correlation between CRH neuron number and the last Cornell score (Table 1). According to the DSM-criteria, depression is considered an on-off phenomenon: either you suffer from MDD or not. At the same time it is known that also ‘minor’ depression, or only some depressive symptoms, can have a major impact on daily functioning in AD (Starkstein et al., 2005).

Historically, the field of psychiatry advanced through serendipitous discoveries, like the observation that the antituberculosis drug iproniazid elevated mood and inhibited monoamine oxidase (Nemeroff et al., 2005). In theory, however, drug therapy is based on the correlation between the actions of a drug and the etiology of the disease it is meant to treat. Preclinical studies of CRH-R1 antagonist, antagonizing the CRH receptor at the level of the pituitary, have demonstrated positive behavioural effects with little or no compromise of HPA-axis function (Nemeroff et al., 2005). In addition, in a clinical study Zobel et al. found significant and dose-related improvements in baseline depression and anxiety rating scale scores when 20 patients with a major depressive episode were treated with R121919, an antagonist of CRH(1)-receptors (Zobel et al. 2000). The CRH(1)-receptor blockade brought about by this CRH(1)-receptor antagonist turned out not to impair the corticotropin and cortisol secretory activity either at baseline or following an exogenous CRH challenge (Zobel 2000). Other CRH-R1 antagonists are under investigation (Nemeroff et al., 2005; Keller et al., 2006). It seems, therefore, possible that the HPA-axis hypothesis, especially the hyperactivity of CRH neurons in the PVN, may lead to the development of antidepressant medication. Based on our results, we could hypothesize that this type of antidepressant would also be beneficial for depressed AD patients.

Chapter 6: Vasopressin and oxytocin in AD
The results of Chapter 2 and 3 concerning the increased OXT and AVP expression in the PVN and SON of depressed patients gave us cause to test the
same parameters in depressed and non-depressed AD patients. In the study of Chapter 6 we measured the amount of AVP mRNA and OXT mRNA using \textit{in situ} hybridization in the PVN and SON in patients from the same cohort of AD patients as used in Chapter 4 and 5 and we used exactly the same parameters for depression as in Chapter 5: DSM-IIIR and the Cornell scale (Table 1). We did not find a significant difference in the amount of AVP or OXT mRNA in the PVN or SON between depressed and non-depressed AD patients. In addition, we found no significant correlation between the Cornell score and the amount of AVP or OXT mRNA in the PVN or SON.

Although we did not find a significant relationship between AVP and OXT mRNA on the one hand and depression in AD on the other, these neuropeptides may play a role in AD. As discussed concerning the results of Chapter 3, where we hypothesized OXT expression to be related to loss of weight in MDD, OXT is a satiety peptide (Gimpl et al., 2001; Meynen et al., 2007a). Loss of weight, and the problems arising from it have been well described in AD (Riviere et al., 1999; Guerin et al., 2005). Accelerated weight loss has been observed even as preceding the clinical diagnosis of AD (Johnson et al., 2006), while Buchman et al. described a declining body mass index (BMI) to be associated with increased risk of incident AD (Buchman et al., 2005). Since loss of appetite has been identified as a factor in weight loss in AD (Wang et al., 2004), it can be hypothesized OXT may be involved in weight loss in AD.

A decrease in pain perception is a consistent finding in AD (Scherder et al., 2003). Both AVP and OXT are known to have an antinociceptive effect (Scherder et al., 2003). Therefore, it could be hypothesized that hypothalamic OXT and AVP contribute to a decrease of pain perception in AD. In addition, CRH neurons in the PVN that produce analgesia (Scherder et al., 2003) are hyperactive in AD (Raadsheer et al., 1995) and these are increased in depression in AD in a way we described in Chapter 5.

Chapter 7: Depression and senile plaques

The previous chapters presented and discussed studies concerning biological parameters in the HPA-axis. This sixth chapter presents the results of a study aimed at determining the relationship between depression and the neuropathological hallmarks of AD: neuritic plaques (NP) and tangles. The positive relationship we found between plaques and the Cornell scores in Chapter 7, raises the question how the plaques could be related to depressive symptoms. We will further (see Chapter 7) address three possible ways in which they may be linked.

First, both the formation of NP and depression could be effects of a common
underlying pathogenetic factor in AD, such as decreased cortical metabolism (Swaab et al., 2002) or vascular factors that are implicated in both AD (Newman et al., 2005) and MDD (Whooley, 2006). Depression is a risk factor for cardiovascular disease (Ferketich et al., 2000; Whooley, 2006), while cardiovascular disease is a risk factor for AD (Newman et al., 2005). Recent regional cerebral blood flow SPECT data provide preliminary anatomical support for the possibility that AD-like brain changes may develop in heart failure patients, possibly as a consequence of chronic cerebral blood flow reductions (Alves et al., 2006). The factor of cardiovascular disease itself may be further analyzed by determining, e.g., the contributions of the separate risk factors for cardiovascular disease, such as hypertension, hyperlipidemia and diabetes mellitus, for depression in AD (Bergmann et al., 2006). Midlife hypertension has been found to be associated with greater prevalence of AD in late-life, the risk being particularly high for those who were not treated (Rosano et al., 2006). Both in depression (Raadsheer et al., 1994a; Raadsheer et al., 1995) and in hypertension (Goncharuk et al., 2002) the number of CRH neurons in the PVN is increased. Carotid wall thickness is a marker for cardiovascular disease (Rosano et al., 2006). To further assess the possibility that cardiovascular disease is an underlying factor for both AD and depression in AD, measurements of the carotid wall thickness in vivo could provide valuable additional information for post mortem brain research. Another factor that should be included is APOE4 allele polymorphism. This polymorphism accounts for about 10% of cholesterol level variation in the population. Two studies have found that AD risk related to cardiovascular risk factors was greater in the presence of APOE4 allele than in the absence of APOE4 (Rosano et al., 2006). Dubelaar et al. observed that APOE4 may act as a risk factor for AD by decreasing neuronal metabolism, since, even in controls that did not show any sign of AD pathology, they found a decrease in neuronal metabolism in those subjects carrying an APOE4 allele (Dubelaar et al., 2004). Until now conflicting results have been found concerning the relationship of APOE4 and depression in AD (Levy et al., 1999; Muller-Thomsen et al., 2002; Craig et al., 2005). In future research of this type, information about the history of cardiovascular disease will be important. Probably more than one of the factors mentioned (decreased cortical metabolism and cardiovascular disease) is present in interaction. The elucidation of the relation between depression in AD and cardiovascular disease, could, given the relationship of MDD and cardiovascular disease, also be of importance for this broader topic of depression and cardiovascular disease.

Second, the presence of NP in the cortex may lead, possibly via toxicity of Aβ-amyloid, to the occurrence of depressive symptoms. Neuronal damage in the temporal lobe could lead to disinhibition of the HPA-axis, since under
physiological circumstances the hippocampus is an inhibitor of HPA-axis activity (Herman et al., 2005) (see Chapter 7).

Third, stress-related disorders, such as depression, are frequently accompanied by hyperactivity of the HPA-axis which, in turn, could contribute to the AD process via deleterious effects on the hippocampus (Sapolsky, 2000). However, Muller et al. found that neither MDD nor glucocorticoid treatment affects the cellular integrity of the human hippocampus (Muller et al., 2001), while Lucassen et al. observed that hippocampal apoptosis in MDD is a minor event and absent from subareas at risk for glucocorticoid overexposure (Lucassen et al., 2001).

Returning to the central issue of this thesis, to what extent MDD and depression in AD share their pathophysiology, we can conclude that although CSF cortisol (Chapter 4) and hypothalamic AVP (Chapter 6) show a difference in depression in AD as compared to MDD, our finding concerning CRH neurons in the PVN indicates a similar pathophysiology of depression in AD and MDD (Table 1). In addition, concerning the noradrenergic system, in previous studies no differences between depressed and non-depressed AD patients were found as far as the total number of norepinephrine-producing locus coeruleus neurons (Hoogendijk et al., 1999b) or measures of metabolic activity of norepinephrine, serotonin, dopamine in the cerebral cortex, hippocampus, amygdala and locus coeruleus were concerned (Hoogendijk et al., 1999a). Regarding the serotonergic system only few studies were performed that compared depressed and non-depressed AD patients, and the results were controversial (Hendricksen et al., 2004). Chapter 7 shows a correlation between the presence of NP and the score on the Cornell scale for depression in dementia. Since neuritic plaques are not present in MDD, at first sight this finding points in the direction of a different pathogenesis of MDD and depression in AD. However, as mentioned above, it might be that the relationship between neuritic plaques and the depressive symptoms is linked to a mechanism shared by both disorders, e.g., HPA-axis activation.

Our initial question, i.e. whether MDD and depression in AD share their pathophysiology, is addressed in the present thesis. Both conditions showed a similar pathophysiological change of increased activity at the level of CRH neurons in the PVN. In addition, our results showed that depressive symptoms in AD are related to the number of neuritic plaques. Regarding AVP and OXT mRNA expression in the PVN and SON, we found no changes in depression in AD, whereas we did find an increased level of AVP mRNA in the SON in MDD. In the melancholic subtype an increase in AVP mRNA was found in both the PVN and the SON. For OXT mRNA, we found an increase in the PVN.
in melancholic type compared to non-melancholic-type depressed patients. Our results draw attention to the possible importance of AVP as a pathophysiological factor in MDD, and to the SON as the main source of this AVP. In addition, our findings provide additional ground for the distinction between melancholic-type depression and non-melancholic-type depression when evaluating molecular changes in the brain. Future research is necessary to further evaluate the pathophysiology of MDD and depression in AD - their similarities and differences - and especially the susceptibility of MDD and depression AD to different treatment strategies.

**Future research**

HPA-axis hyperactivity in depression is well established, and important regulating components of this axis have been identified. In addition, certain systems, like the GABAergic system (Giordano et al., 2006), are known to influence the HPA-axis. At the same time there will be other neurotransmitters and neuropeptides that influence the HPA-axis, whose influence is not yet known. Further research should bring enlightenment. This means that, while hypotheses concerning known factors (CRH, OXT, AVP) have to be tested, at the same time new hypotheses will have to be generated. This could be accomplished via an approach in which, on the one hand (hypothesis generating) new genes involved in depression and HPA-axis regulation are identified by micro-array technology, while on the other hand (hypothesis driven) in there will be a focus on the already identified factors, like AVP, CRH and OXT. These kinds of experiments could initially be performed in living subjects: 1) in peripheral blood samples of (well-characterized) depressed subjects or depressed AD patients, or 2) in animal models of depression and AD. When certain genes (peptides) are identified, the results could then be validated in human post mortem studies. At the same time, when certain factors are found to be correlated in human post mortem studies, the causal relationship between these factors can be studied in animal experiments.

**Reference List**


Chapter 8


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