Plasma cortisol and norepinephrine in Alzheimer's Disease. Opposite relations with recall performance and stage of progression

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Acta Neuropsychiatr 2007, 19, 231-237
Abstract
Alzheimer’s disease (AD) is characterized by effortful retrieval memory impairments, loss of hippocampal neurons, and elevated plasma cortisol (CORT) concentrations. The latter could induce further memory decline. AD is also characterized by increased central and peripheral noradrenergic activity. Since noradrenergic function is involved in memory formation, this upregulated function could counteract memory decline. The aim of the present study was to test these hypotheses using plasma norepinephrine (NE) as noradrenergic parameter, and recall of the prerecency part of neutral valence word lists as measure of effortful retrieval.
Area under the curve (AUC) of morning, midday and afternoon plasma CORT and plasma NE concentrations were related to two measures of recall performance, i.e. summated recall scores of the prerecency and recency parts of three word lists, and to the stage of the Clinical Dementia Rating (CDR).
Partial correlation between each hormone AUC value with prerecency recall performance, controlling for the effect of the other hormone, showed opposite relations between recall and either plasma CORT or NE. Similar more strong correlations were found with the CDR score.
Plasma CORT and NE are oppositely related with effortful retrieval and the stage of progression in AD.
Introduction

Alzheimer’s disease (AD) is characterized by episodic memory impairments in the early course of the disease [19]. One of the frequently used techniques to assess episodic memory has been the use of free recall of word lists. By means thereof it has been repeatedly demonstrated that the onset of the disease is characterized by recall impairments of the first few words in a list (also known as prerecency part) [3,4,13,15], which has been found associated with effortful memory retrieval [16,23] and hippocampal damage [17]. As the disease progresses recall impairment of the last few words in a list (also known as the recency part) has also been found [3,4,13,15].

AD is further characterized by loss of hippocampal neurons in the early onset of the disease [28], elevated plasma CORT concentrations and both elevated cerebrospinal fluid (CSF) NE [12,29] and plasma NE concentrations [12].

According to the glucocorticoid cascade hypothesis [32] the hippocampal cell loss in AD would result in hypercortisolism that in turn would act as a co-factor in further hippocampal degeneration. Yet no evidence for this has been found in ageing or AD [26, 37]. Instead, in rodents evidence has been found of a reversible negative influence of hypercortisolism on the hippocampus [37]. In particular it has been found that hypercortisolism results in dendritic remodelling and a suppression of neurogenesis in the dentate gyrus that normalize when CORT concentrations normalize [14]. In addition it has been found that hypercortisolism is mild in AD and occurs only in half of the cases [25,37]. Moreover, hippocampal damage does not inevitably lead to hypercortisolism [9]. Hypercortisolism in AD therefore appears to be not related to hippocampal cell loss, but may nonetheless have reversible negative effects on hippocampal function. This predicts that in AD memory performance on the prerecency part is inversely related to plasma CORT. Since this memory deficit is a core feature of the clinical picture of AD, the stage of progression of AD could similarly be related to plasma CORT.

Evidence is also mounting that there is a significant abnormality in the noradrenergic system in AD [17]. CSF NE concentrations but also plasma NE concentrations have been
found to increase in AD [12,29]. This increase occurs after locus ceruleus (LC) noradrenergic neuronal loss in AD [8]. One interpretation, based on animal studies [1], has been that increased activity of the surviving central nervous system (CNS) neurons in response to partial LC damage is a compensatory activity. Three studies have reported evidence for this in man. Two studies found an inverse association between central NE concentrations and LC cells in AD [20,29]. One study found a direct association between the peripheral noradrenergic marker plasma 3-methoxy-4-hydroxyphenylglycol (MHPG) and cognitive impairment in AD [24]. Since NE function is also involved in memory formation [31], this upregulated function could counteract memory impairments. As plasma NE has been found related with central NE function [40], we used this noradrenergic parameter in the present study. Analogous to the CORT hypothesis, the second hypothesis of the present study therefore was that in AD both memory performance on the prerecency part and stage of progression are inversely related to plasma NE.

Material & Methods

Subjects

AD patients living in a nursing home or at home were recruited via a regional data bank. The age-matched normal control (NC) group consisted of partners or acquaintances of patients in the nursing home or at home. All participants were informed of the goal of the study. Written informed consent was obtained. The Leiden University Medical Ethics Committee approved this study.

Diagnosis

For inclusion in the study a complete medical history was obtained for all participants. Subsequently they received a physical examination by the second author, and were excluded if suffering from any other neurological disturbance, or systemic disorder, or were taking lithium, calcium antagonists, anti-arhytmatica, or benzodiazepines within
three weeks before testing.
Diagnosis was checked using the criteria of the fourth edition of the Diagnostic and Statistical Manual (DSM-IV) [2]. Clinical severity was rated by means of the Clinical Dementia Rating (CDR) [21] (see table 1).

Methods
Before memory performance was assessed at approximately 1600 h, daytime CORT and NE activity was assessed using blood samples obtained at 0900, 1200 and 1600 h from all participants. This was done to rule out possible confounding effects of the testing condition on baseline neuroendocrinological activity. Confounding effects of venipuncture on baseline neuroendocrinological activity were regarded as negligible as the correlation between saliva CORT and plasma CORT has been found to be .96 in the elderly [38]. All subjects were tested in the nursing home or at home.

Blood samples
Within 1 hour after venipuncture plasma was separated and frozen at -20° C until used. The CORT measurement was based on competition for CORT specific antibody between a known amount of labelled CORT and endogenous CORT in the sample. It was measured using a commercial available kit (Boehringer Mannheim, Germany, Order No. 1 098 578) and performed on an ES 300 (Boehringer Mannheim, Germany). Plasma NE was extracted binding it to aluminum oxide, and its concentration was determined by means of high performance liquid chromatography (HPLC) using electrochemical detection, with dihydroxybenzyllamine as an internal standard[22].

Memory assessment
Memory was assessed by means of a modified version of the Rey Auditory Verbal Learning Test (RAVLT) [4,30]. Preceding five presentations and immediate free-recall of a 15-word list, which is the standard presentation of the RAVLT, immediate recall of five presentations was asked of the first six words in the list and, subsequently, of the
remaining nine words of the list. Words were read at a speed of 1.5 s per word, and always presented in the same order. Recall was oral.

Recall of the lists was transformed into serial position curve (SPC). The reason for this analysis was because two functions arise from the SPC, which we previously demonstrated [6,7]. One function underlies the recency part i.e. the last two to three items in a list, while the other function underlies the primacy and middle (prerecency) part i.e. the remaining items in a list.

**Raw item scores**

Percentages were calculated of the summated free recall over five trials of the three prerecency parts (hereafter denoted as Pre6, Pre9, Pre15). Similarly, percentages were calculated for the three recency parts (hereafter denoted as Rec6, Rec9 and Rec15). In addition, the percentages of the three prerecency and recency parts were summated (hereafter denoted PreT and RecT).

**Weighted item scores**

The weighted item scores were computed by multiplying the raw item scores by the corresponding factor coefficient scores [6]. Subsequently, similar calculations were performed on the raw item scores for the three prerecency parts (hereafter denoted as facPre6, facPre9, facPre15), the three recency parts (hereafter denoted facRec6, facRec9, facRec15) and summated scores of the three prerecency and recency parts (hereafter denoted facPreT and facRecT).

**Statistical analysis**

Data were verified for assumptions of normality and sphericity, and logarithmic transformations and Greenhouse & Geisser corrections were applied when normality or sphericity was not met.

Pearson correlational analysis was performed between the raw and weighted item
scores, CDR, and hormonal measures. Two-tailed levels of significance were applied. Comparability of the experimental groups regarding demographic and clinical variables was tested using one-way analysis of variance.

In order to evaluate specific effects of the various hormonal and recall measures on group performance, multivariate analysis of variance (MANOVA) with repeated measures of design with or without covariate was used.

As a nominal level of significance, $\alpha = 0.05$ was accepted. Analyses were performed using statistical software programs (SPSS for Windows 9.0, SPSS Inc. Chicago, Ill. U.S.A.)

**Results**

*Demographic and clinical data*

There were 22 AD patients and 21 NC participants. However, one AD patient had to be excluded because of lack of memory assessments. The demographic data of the two groups are described in table 1.

**Table 1** Demographic data of the Alzheimer disease (AD) and Normal control (NC) group.

<table>
<thead>
<tr>
<th>Scale</th>
<th>AD</th>
<th>NC</th>
<th>F-score</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>85.2 ± 5.1</td>
<td>85.7 ± 6.4</td>
<td>.08</td>
<td>.77</td>
</tr>
<tr>
<td>Sex</td>
<td>3M, 18F</td>
<td>8M, 13F</td>
<td>3.2</td>
<td>.08</td>
</tr>
<tr>
<td>Level of education</td>
<td>8.5 ± 0.9</td>
<td>9.2 ± 2.8</td>
<td>1.29</td>
<td>.26</td>
</tr>
<tr>
<td>CDR</td>
<td>1.6 ± 0.7</td>
<td>0.01 ± 0.2</td>
<td>102.5</td>
<td>.000</td>
</tr>
</tbody>
</table>
As can be read from this table the groups did not differ in age, sex, and level of education, but did differ in CDR score. NE could be determined in 19 AD patients (3M, 16F, age 85.2 ± 5.3 yrs), and 10 control subjects (3M, 7F, age 85.9 ± 6.3 yrs).

Recall in AD and controls

Mean percentages of the summated raw item scores are depicted in fig 1 a, b. Both types of recall scores showed a normal distribution so that a normalizing transformation was not necessary.

Table 2 Mean and standard deviations of the daytime and area under the curve (AUC) values of plasma cortisol (CORT) (nmol/l) and plasma norepinephrine (NE) (nmol/l) concentration levels in Alzheimer disease (AD) and normal control (NC) group.

<table>
<thead>
<tr>
<th></th>
<th>AD (Mean ± S.D.)</th>
<th>NC (Mean ± S.D.)</th>
<th>F-test</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n= 21)</td>
<td>(n=21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CORT9</td>
<td>634 ± 240</td>
<td>491 ± 121</td>
<td>2.9</td>
<td>.10</td>
</tr>
<tr>
<td>CORT12</td>
<td>482 ± 197</td>
<td>308 ± 100</td>
<td>12.6</td>
<td>.001</td>
</tr>
<tr>
<td>CORT16</td>
<td>417 ± 200</td>
<td>304 ± 89</td>
<td>3.3</td>
<td>.08</td>
</tr>
<tr>
<td>CORTAUC</td>
<td>3457 ± 1297</td>
<td>2421 ± 513</td>
<td>9.0</td>
<td>.006</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>AD (N=19)</th>
<th>NC (N=10)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>NE9</td>
<td>5.38 ± 1.87</td>
<td>4.69 ± 2.1</td>
<td>.91</td>
<td>.37</td>
</tr>
<tr>
<td>NE12</td>
<td>5.69 ± 2.05</td>
<td>4.29 ± 2.2</td>
<td>1.72</td>
<td>.10</td>
</tr>
<tr>
<td>NE16</td>
<td>5.09 ± 2.44</td>
<td>4.69 ± 3.1</td>
<td>.38</td>
<td>.71</td>
</tr>
<tr>
<td>NEAUC</td>
<td>38.17 ± 13.48</td>
<td>31.44 ± 15.90</td>
<td>1.45</td>
<td>.24</td>
</tr>
</tbody>
</table>
MANOVA with repeated measures design of the percentages of the summated raw item scores revealed a significant main effect of prerecency (F(2,63)= 52.4, p<.001), and a significant group difference on prerecency (F(1,37)=35.9, p<.001), but no significant x prerecency interaction (F(2,63)=2.0, p=.15). It further revealed a significant main effect of recency (F(2,73)= 39.3, p<.001), a significant group difference on recency (F(1,37)= 12.4, p=.001), while no significant group x recency interaction was found (F(2,73)= 1.4, p=.25).

MANOVA with repeated measures design of the percentages of the summated weighted item scores of prerecency (facPreT) and recency (facRecT) recall showed a significant main effect of prerecency (F(2,56)= 27.8, p<.001), a significant group difference on prerecency (F(1,37)= 42.9, p<.001), and a significant group x prerecency interaction (F(2,56)= 26, p<.001). It further revealed a significant main effect of recency (F(2,67)= 23.6, p<.001), a significant group difference on recency (F(1,37)= 5.3, p=.027), while no significant group x recency interaction was found (F(2,67)= 2.1, p=.14). In the subsequent analyses only facPreT and facRecT were used.

**Table 3** Pearson correlation between summated prerecency (facPreT) and recency (facRecT) parts, the CDR score, area under the curve cortisol values (**CORTAUC**), area under the curve norepinephrine (**NEAUC**) values, and p-value (italicized) in the Alzheimer disease (AD) and Normal control (NC) group.

<table>
<thead>
<tr>
<th></th>
<th>AD</th>
<th>NC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CORTAUC</td>
<td>NEAUC</td>
</tr>
<tr>
<td>facPreT</td>
<td>-.30</td>
<td>.19</td>
</tr>
<tr>
<td></td>
<td>.04</td>
<td>.44</td>
</tr>
<tr>
<td>facRecT</td>
<td>.14</td>
<td>.10</td>
</tr>
<tr>
<td></td>
<td>.55</td>
<td>.70</td>
</tr>
<tr>
<td>CDR</td>
<td>.53</td>
<td>-.16</td>
</tr>
<tr>
<td></td>
<td>.01</td>
<td>.53</td>
</tr>
</tbody>
</table>
The CDR score was found to correlate to facPreT (r = .80, <.001) and to facRecT (r = .38, p = .018) in the groups combined.

**Stress-hormone concentrations**

The CORT and NE values are presented in table 2. The three CORT and NE values showed a normal distribution. MANOVA with repeated measures design using the neuroendocrinological values as dependent measures showed a main effect of CORT (F(2,75) = 42.7, p < .001), no group x CORT interaction (F(2,75) = .85, p = .42), but a significant group difference on CORT (F(1,40) = 10.6, p = .002). With regard to NE no main effect (F(2,42) = .12, p = .84), no significant group x NE interaction (F(2,42) = 1.3, p = .27), and no significant group difference on NE (F(1,27) = 1.1, p = .31) were found.

**Table 4** Partial correlation between summated prerecency (facPrecT) , the CDR score and CORTAUC and NEAUC, using either hormone as control variable, and p-value (italicized) in the Alzheimer disease (AD) group.

<table>
<thead>
<tr>
<th></th>
<th>AD</th>
<th>NEAUC (CORTAUC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>facPreT</td>
<td>- .51</td>
<td>.46</td>
</tr>
<tr>
<td></td>
<td>.04</td>
<td>.06</td>
</tr>
<tr>
<td>CORT</td>
<td>.66</td>
<td>- .53</td>
</tr>
<tr>
<td></td>
<td>.003</td>
<td>.03</td>
</tr>
</tbody>
</table>

As a consequence thereof area under the curve (AUC) values were used in the subsequent analyses. Since the intermediate time periods between the blood samples were three and four hours, area under the curve (AUC) values were calculated for CORT and NE (hereafter denoted CORTAUC and NEAUC) using the formula 3 x
CORT12 + 4 x CORT16 + 3/2 (CORT9 – CORT12) + 2 x (CORT12 – CORT16) and 3 x NE12 + 4 x NE16 + 3/2 (NE9 – NE12) + 2 x (NE12 – NE16).

Interaction between recall and stress hormones in AD and controls
MANOVA with repeated measures of design of the percentages of the summated weighted item scores of prerreency and recency recall using CORTAUC as covariate showed that memory performance on the prerreency part interacts significantly with CORTAUC (F(2,56) =3.7, p=0.041), but not memory performance on the recency part (F(2,65) =1.1, p=0.34).

Correlation between recall and stage of progression and the stress-hormones
Correlational analysis between prerreency, CDR and CORTAUC is depicted in table 3. Since in the AD group CORTAUC and NEAUC were found to correlate (r= .51, p=0.03), suggesting that they could interact when influencing memory performance, partial correlation was calculated.

Figure 1 a, b. Mean percentages of the summated raw item scores and standard error of measurement (S.E.M) of the prerreency part (Pre) (a) and the recency part (Rec) (b) of the three lists in Alzheimer’s disease (AD) patients and normal control (NC) participants.
As is shown in table 4 the correlations between facPreT and CDR on the one hand and CORTAUC and NEAUC on the other, increased in strength when either hormone was used as control variable. The increased strength in the correlations presented in Table 4 compared to Table 3 is not simply due to the fact that the number of subjects is smaller. There were no significant correlations between prerecency recall or CDR and CORTAUC in the normal control group.

**Discussion**

We found that plasma CORT and plasma NE are oppositely related to memory performance on the prerecency part. As memory performance on that part has been associated with effortful retrieval [16,33] and hippocampal function [17], these results support the hypothesis that these hormones are oppositely related to effortful retrieval and hippocampal function in AD. The fact of strongest relations being found when using the factor-analytically derived prerecency recall scores [6] supports the usefulness of this method. In addition, we found similar opposite relations between these stress-hormones and the stage of AD progression (CDR), and these relations could not be explained by age.

However, the opposite relations of these stress-hormones are cognitively specific. The stage of progression (CDR) is explained by 64% of the variance of the prerecency part, but only 16% of the variance of the recency part, and thus also predominantly a measure of effortful retrieval.

The specificity of the relations between plasma CORT en NE and effortful retrieval in AD becomes clear when contrasted with findings in elderly depressed [4] and middle-aged healthy subjects [7], where the same design was used. In both groups it was found that non-elevated plasma CORT concentrations are directly related to effortful retrieval and that in the group of middle-aged healthy subjects non-elevated plasma NE concentrations are inversely related to effortful retrieval. In combination with the present findings these CORT related results are supportive of the general claim that moderate levels of glucocorticoids promote and high levels inhibit cognitive
The NE results support the hypothesis that enhanced NE function promotes cognitive performance. The present and previous findings [7] imply that peripheral NE concentrations either represent central noradrenergic function as has been demonstrated [40], and/or that they may influence hippocampal function indirectly. With regard to the latter and recognizing the well-established fact that plasma NE does not pass through the blood-brain-barrier [39], this effect could be directed via the vagus nerve, tractus solitarius, LC, and basolateral amygdala (BLA) [10]. In support of this pathway vagus nerve stimulation has been found to improve cognitive performance in AD [35]. Stimulation of the noradrenergic system by means of pharmacological manipulations has demonstrated an enhancing effect of noradrenergic activation on long-term memory for emotionally arousing information [27, 36]. The results of the present study suggest that this enhancing effect extends to neutral valence words. Unexpectedly, no associations were found between CORT or NE and effortful retrieval in the normal control group. Previous studies have found associations between increased basal CORT levels and declines in cognitive abilities, most notably episodic memory, in cross-sectional and longitudinal studies in normal controls [5,22,34]. Plasma NE has also been found related to cognitive performance in healthy controls [7]. A possible explanation why we did not find these relations may be due that the normal control group of the present study was small, and older than the normal controls investigated thus far. Next to a group difference in effortful retrieval, we further found a group by effortful retrieval interaction. This indicates that AD patients differ not only quantitatively but also qualitatively in their ability to deploy effortful retrieval over the three lists presented from the NC group. In combination with the opposite associations found between these stress-hormones and this measure, it is suggested that elevated CORT levels have a negative effect on this type of memory performance, which is counteracted by elevated NE levels. Hypercortisolism has been found to result in dendritic remodelling and a suppression
of neurogenesis in the dentate gyrus that normalize when CORT concentrations normalize [14]. Although further research is needed, a tempting thought is that elevated CORT levels in AD are indicative of dendritic remodelling and a suppression of neurogenesis, for then treatment of memory impairments may become an option.

Acknowledgements
We would like to thank Drs K.P.M. van Wijk-Kerssemakers, F. Tabeling, R. Jonquière, H. van Andel for supplying the AD patients, Mrs. J. Schouten-Verhagen, Miss J. Schouten, and J. Turfboer for the lab measurements, and Prof Dr A.H. Zwinderman and Dr. B.Mertens for their statistical advice.
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