Stress, the brain and cognition

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Stress, the brain and cognition

Stress, het brein en cognitie

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Chapter 3

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Chapter 4

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Contents

Chapter 1

General Introduction

1

General introduction

As the population is growing older, the risk for neurodegenerative disorders, like Alzheimer's disease, increases. Currently, the lifetime cumulative risk for developing dementia is 20 % and with increasing life expectancy it is estimated that the number of affected people over the next half-century may be as high as 50 % in the developed countries.[1;2] Alzheimer's disease (AD) is the most common type of dementia, and is characterized by cognitive decline, medial temporal lobe atrophy and various behavioural problems. For instance, 30 to 50 % of patients with AD suffer from depression,[3;4] leading to more functional disability, worsening of cognitive deficits and extra burden for the caregiver. It remains however unknown whether depression is an early symptom of neurodegeneration, whether it is a psychological reaction to early cognitive deficits, or whether depression represents a risk factor for dementia.[5-8] At least three possible explanations have been proposed, namely (a) depression is a prodrome or early symptom of dementia; (b) depression and dementia are independent illnesses, which co-occur coincidentally; and (c) depression leads to increased levels of glucocorticoids, which causes damage to the hippocampus and eventually dementia.[9] The latter explanation is based on the glucocorticoid cascade hypothesis [10] and will be investigated in this thesis.

Glucocorticoid cascade hypothesis

When an event is interpreted as being stressful, it triggers the activation of the hypothalamic-pituitary-adrenal (HPA) axis. The end product of this activation is the secretion of glucocorticoids by the adrenal cortex; in humans the main glucocorticoid is cortisol. While the activation of the HPA axis can be regarded as a basic adaptive mechanism in response to change, prolonged activation has been associated with a variety of illnesses, such as hypertension, diabetes mellitus, arterial disease and neuropsychiatric disorders, like depression and Alzheimer's disease.[11-17]

According to the glucocorticoid cascade hypothesis, chronic exposure to glucocorticoids can lead to hippocampal volume loss, which then may result in a diminished inhibitory feedback to the HPA axis [18] This impaired inhibitory effect may result in hypersecretion of cortisol, which, in turn, may result in further neuronal loss of the hippocampus (see figure 1).[19] Since the hippocampus is important for memory function and is one of the first brain structures showing atrophy in AD, it has been hypothesized that depression and stress can lead to AD, via chronic exposure to elevated levels of cortisol.

So far, this hypothesis has mainly been tested in experimental settings (e.g. animal studies and stress-induced studies) and based on these studies it has become clear that in some cases the detrimental effects of stress and depression are reversible [20-22] and that not only the hippocampus is a target, but the amygdala [23] and the

frontal cortex as well.[24] Moreover, recently it has been demonstrated that particularly in case of acute stress the negative feedback regulation of the HPA-axis depends on glucocorticoid receptors in the hippocampus,. [25] whereas in case of chronic stress it has been suggested that negative feedback regulation depends on other structures, such as the amygdala.[26] Also, several studies have reported that smaller hippocampal volumes can be a risk factor for the development of post-traumatic stress disorder[27] and depression[28] rather than a consequence of these stress-related disorders. Nonetheless, the popularity of the glucocorticoid cascade hypothesis as an explanation for the co-occurrence of depression and AD is still persistent in clinical research but has hardly been tested in large population-based studies.

General objective

The aim of this thesis was to investigate the relations between stress, depression and early symptoms or preclinical markers of AD, like cognitive decline and volume loss of the hippocampus and entorhinal cortex, and to investigate to which extent these relations are explained by the glucocorticoid cascade hypothesis.

Study populations

Data from two large cohort studies were used, namely from the Longitudinal Aging Study Amsterdam (LASA) and the Second Manifestations of ARTerial disease-Memory, depression and aging (SMART-Medea) study.

LASA is a population-based prospective cohort study among 3,107 persons initially aged 55–85 years in the Netherlands that started in 1992/1993. Details of this cohort study have been described previously.[29] For this thesis, data were used from the third follow-up in 2001-2002 when 1,691 subjects participated (mean age 75±7 years; 46 % male) and the fourth follow-up in 2005-2006 when 1,266 subjects participated (mean age 78±7 years; 43 % male).

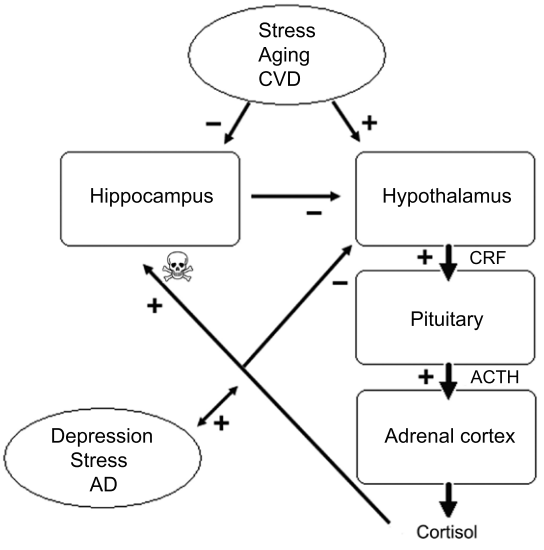
The SMART-Medea study is an ancillary study to the SMART-MR study[30;31] aimed to investigate brain changes associated with psychosocial vulnerability and stress factors in independently living patients with symptomatic atherosclerotic disease. For this thesis data were used from the measurement in 2006 – 2009, when 754 patients participated (mean age 61±9 years; 81 % male).

So far, most preceding studies were conducted in younger adults. We chose to conduct the studies in LASA and SMART-Medea because older persons and persons with atherosclerotic disease may be more vulnerable for HPA axis dysregulation and neurodegeneration.

Outline

In the first two chapters psychosocial determinants of HPA axis regulation in older persons are investigated. In chapter 2.1 the personality characteristics neuroticism, self-esteem and mastery are investigated in relation to salivary morning and evening cortisol. In chapter 2.2 the single and combined effects of early and late life events on HPA axis regulation are investigated. In the following three chapters the possible detrimental effects of stress and HPA axis activity on cognitive impairment and decline are investigated. In chapter 3.1 the longitudinal association between serum cortisol levels and cognitive decline in older persons is described. Chapter 3.2 describes the longitudinal relation between salivary cortisol levels, apolipoprotein E4 allele and cognitive decline in older persons and in chapter 3.3 the relation between the experience of life events, salivary cortisol and cognitive functioning in patients with arterial disease is described. In the last two chapters, the relation between HPA axis dysregulation, depression and medial temporal lobe volumes is investigated. In chapter 4.1 the relation between HPA axis regulation and hippocampal volume in patients with arterial disease is described. In chapter 4.2 we firstly investigated the relation between depression and hippocampal and entorhinal cortex volumes in patients with arterial disease. Secondly, we examined the role of HPA axis regulation in the association between depression and hippocampal and entorhinal cortex volumes. Based on our results we will discuss in chapter 5 whether the glucocorticoid cascade hypothesis holds true and the findings of the different studies in this thesis are summarized in chapter 6.

Figure 1. Schematic overview of glucocorticoid cascade hypothesis.



CVD: cardiovascular diseases
CRF: corticotropin releasing factor
ACTH: adrenocorticotropic hormone
AD: Alzheimer's disease

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Chapter 2

Psychosocial determinants of HPA-axis activity

2

2.1 Personality characteristics and hypothalamic-pituitary-adrenal axis regulation in older persons

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Abstract

Objective

To investigate the cross-sectional association between personality characteristics and hypothalamic-pituitary-adrenal (HPA) axis regulation in older persons.

Methods

The study sample consisted of 1,150 participants (mean age 74.8 ± 7.1 years, 48% male) from the population-based Longitudinal Aging Study Amsterdam (LASA). HPA axis activity was measured with salivary cortisol collected after awakening and late in the evening. Outcome measures were awakening and evening cortisol levels (natural log-transformed) and the diurnal pattern of cortisol. Determinants were scores on questionnaires assessing neuroticism, mastery, and self esteem.

Results

Multiple linear regression analyses adjusted for potential confounders did not show significant associations between any of the personality characteristics and any of the cortisol measures. On evening cortisol, a significant interaction was observed between neuroticism and age (B = -0.001; T = -2.50; df = 1139; p-value = 0.01). After stratification in two age groups we observed that high levels of neuroticism were associated with elevated levels of evening cortisol in subjects aged <75 years (B = 0.02; 95 % CI: 0.01 to 0.03; T = 2.15; df = 630; p = 0.03), but not in subjects aged 75 or older.

Conclusions

The findings of this large population-based study of older persons suggest that the personality characteristics mastery and self-esteem are not associated with HPA axis regulation as measured with salivary awakening and evening cortisol. However, high neuroticism may be associated with elevated levels of evening cortisol in the younger old, but not in the older old.

Introduction

Personality characteristics, like neuroticism and a low sense of mastery, increase the vulnerability to stress related disorders such as depression and posttraumatic stress disorder (PTSD) [1-3]. Depression and PTSD are associated with hypothalamic-pituitary-adrenal (HPA) axis dysregulation [4-6]. It has been suggested that personality characteristics are also associated with alterations in HPA axis regulation.

Studies on the association between personality characteristics, including neuroticism and self-esteem, and HPA axis regulation have mostly been conducted among younger adults and reported inconsistent results [7-14]. Some studies observed lower levels of free cortisol in high neurotic subjects than in low neurotic subjects [7, 9], whereas other studies found an increased cortisol (awakening) response in high neurotic subjects [13, 14] or no associations between neuroticism and HPA axis regulation [8, 10]. In addition, one study investigated the association between free basal cortisol and self-esteem [15], whereas two other studies used a psychosocial stress or cortisol reactivity test to study HPA axis regulation in relation to self-esteem [11, 12]. Two of these studies reported that higher cortisol levels were associated with higher self-esteem whereas the other found that higher cortisol levels were associated with lower self-esteem [12].

HPA axis regulation is also affected by aging [16] and it is therefore of interest to study the association between personality characteristics and HPA axis regulation in older age. So far, three small studies have been conducted in older persons, in which the relation between HPA axis regulation and self-esteem was investigated [17-19]. These studies showed that low self-esteem was associated with elevated cortisol responses after stress [19] and elevated levels of awakening cortisol [17, 18]. So far, no large population-based cohort studies in older persons have examined the relation between different personality characteristics and HPA axis regulation.

The objective of this study was to investigate the association of mastery, neuroticism and self-esteem with HPA axis regulation in a large population-based cohort of older persons. We examined neuroticism, self-esteem and mastery, because self-esteem and mastery play an important role in the perception and interpretation of stressors, whereas neuroticism is considered to be a strong risk factor for onset and course of depression [20-22]. HPA axis regulation was determined by awakening and evening saliva samples; in order to assess the basal circadian rhythm of cortisol.

Methods

Subjects

Data were derived from the Longitudinal Aging Study Amsterdam (LASA). Details of this cohort study have been described previously [23]. For the present study data from the fourth follow-up of 2001-2002 were used, when 1,474 participants underwent a face-to-face interview. Of these, 1,184 participants collected saliva for cortisol measurements. Participants who used corticosteriods were excluded (n=34). We imputed cortisol levels below the detection limit (1.5 nmol/L) with random values between 0 and 1.5 (4 awakening levels and 92 evening levels). Cortisol levels that were considered improbable (>100 nmol/L, 10 morning and 5 evening samples) and evening levels > 5 nmol/L higher than the wakening level (22 samples) were recoded to missing values. The cut-off of 100 nmol/L was chosen rather arbitrarily [24] and corresponds to a cut-off score of 4 SD above the mean awakening cortisol level. Missing values existed also in 9.8 % of the covariates. We used single imputation techniques to impute missing data [25, 26]. Thus, the analytical sample consisted of 1,150 participants.

Measurements

Material for collection of saliva and detailed instructions were sent home to the participants, and the samples were kept in the refrigerator until collected by the visiting nurse the next day. Participants were instructed to collect saliva within 30 minutes after wakening and just before going to sleep in the evening.

Cortisol levels (nmol/L) were determined using radio immunoassay coated tubes (Spectria Orion Diagnostics, Finland). The distributions of awakening and evening cortisol were skewed, therefore natural logarithmic transformations were applied to normalize the distributions. Diurnal variability was calculated as ((awakening cortisol – evening cortisol) / awakening cortisol)*100, to represent the relative decrease of evening cortisol level compared to awakening cortisol level.

In LASA, data was available on the following personality characteristics: neuroticism, self-esteem and mastery. Neuroticism was assessed using an abbreviated subscale of the Dutch Personality Questionnaire (DPQ) [27]. Scale scores range between 0 and 50. Mastery was measured by means of an abbreviated Dutch version of the Pearlin Mastery Scale (PMS) [28] and concerns the extent to which individuals consider themselves to be in control of their own lives; scores range between 5 and 25. Self-esteem was assessed using a shortened version of the Rosenberg Self-esteem Scale, which consisted of 4 items [29]. An example of which is 'On the whole I am satisfied with myself'; the scores can range between 4 and 20. Self-esteem and mastery are thought to be highly correlated [12], however in our study the correlation was below 0.5 and therefore we decided that these measures could be considered as independent personality characteristics.

Age, gender and the highest level of education attained were recorded in the first wave of LASA (1993). Presence of diabetes and history of cardiac diseases were assessed by self-report and medication use. Medication use was verified by the inspection of medication containers by the visiting nurse. Blood pressure was measured twice, and the average of the two measurements was calculated. Hypertension was defined as a diastolic tension above 90 mmHg or a systolic tension above 140 mmHg. Body Mass Index (BMI) was calculated from measured weight and height. Alcohol consumption and smoking status were based on self report. Respondents were also asked to determine the average number of hours of sleep per night.

Data analysis

Firstly, Spearman correlations coefficients were calculated between all independent variables. Secondly, analysis of variance was used to estimate the cortisol levels per quartile of personality measures. For this purpose personality measures were divided into quartiles and were entered as independent variables; natural log-transformed cortisol measures were entered as dependent variables. From the estimated means figures were created, by transforming the cortisol measures back to a linear scale.

Thirdly, all-in multiple linear regression analysis was used to estimate the association between neuroticism, mastery, and self-esteem (all on a continuous scale), as the independent variables, with awakening and evening levels of cortisol and diurnal variability of cortisol, as the dependent variables. Separate models were used to study each personality construct. In the first model no adjustments were made; in the second model adjustments were made for age, sex and level of education and in the third model additional adjustments were made for smoking habits, alcohol consumption, BMI, hypertension, diabetes, cardiovascular diseases, and average number of hours of sleep. We selected covariates a priori and based on the literature [30-33].

Additionally, to investigate whether age affected the relation between personality and cortisol levels we added interaction terms between age (as a continuous variable) and all personality characteristics (as a continuous variable) to the fully adjusted models. Finally, we repeated the analyses after dichotomizing the personality characteristics at the highest quartile of neuroticism score and the lowest quartiles of mastery and self-esteem scores. All analyses were carried out in SPSS version 14.0 (Chicago, Ill, USA).

Results

Table 1 shows the baseline characteristics of the study sample. The mean age of our study sample was 74.8 ± 7.1 years, and 47% was male. The median awakening cortisol level was 15.8 nmol/L (10-90%: 7.4-26.0 nmol/L) and the median evening cortisol level was 4.0 nmol/L (10-90%: 1.6-6.3 nmol/L).
Table 2 gives a correlation matrix of all independent variables. As can be seen, the correlation between the personality characteristics varied between 0.46 and 0.47.

Table 1. Baseline characteristics

Study sample (N)	1,150
Male (%)	47
Age[a]	74.5 (7.1)
Level of education[b] (%)	
- Low	27
- Intermediate	49
- High	14
BMI[a]	27.5 (4.2)
Cardiovascular disease (%)	39
Hypertension (%)	32
Diabetes (%)	10
Current smoker (%)	15
Alcohol consumption (%)	
- no consumption	19
- < 14 drinks/week	59
- ≥ 14 drinks/week	22
Hours of sleep[a]	7.3 (1.4)
CES-D score (0-60) [c,d]	7.0 (1.0-18.0)
CES-D score ≥ 16 (%)	14.6
DPQ Neuroticism score (0-30)[c]	4.0 (0.0 -13.0)
Pearlin Mastery scale (5-25)[a]	17.3 (3.4)
Rosenberg Self-esteem scale (4-20)[a]	15.2 (2.3)
Awakening cortisol [c]	15.8 (7.4 – 26.0)
Evening cortisol [c]	4.0 (1.6 -6.3)

[a]: Data presented as means with sd.
[b]: Low (elementary education or less <= 6 years of education); Intermediate (general education, intermediate vocational education, lower vocational education); High (university education, college education and higher vocational education).
[c] : Data presented as median (range 10-90 %), because of skewed distribution
[d]: Measured with Center for Epidemiological Studies Depression Scale.

Table 2. Correlation matrix of independent variables

	a	b	c	d	e	f	g	h	i	j	k	l	m	n
a) Age	1													
b) Sex (female =0; male =1)	-0.03	1												
c) Level of education	-0.12**	0.25**	1											
d) Body mass index	0.01	-0.14**	-0.18**	1										
e) Cardiovascular diseases	0.14**	0.14*8	-0.06	0.06	1									
f) Hypertension	0.01	-0.09*	-0.04	0.22**	0.14**	1								
g) Diabetes	-0.02	0.00	-0.03	0.14**	0.04	0.12**	1							
h) Current smoker	-0.11**	0.12**	0.02	-0.08*	-0.02	-0.04	-0.01	1						
Alcohol consumption														
i) no consumption	0.12**	-0.15**	-0.17**	0.01	0.06	0.01	0.06*	-0.07*	1					
j) ≥ 14 drinks/week	-0.11**	0.22*	0.15**	-0.03	-0.02	-0.05	-0.03	0.15**	-0.26**	1				
k) Hours of sleep	-0.08**	0.17**	0.10*	-0.03	-0.03	-0.01	0.05	0.08*	-0.00	0.04	1			
l) DPQ Neuroticism score[a]	0.07*	-0.12**	-0.11**	0.03	0.07*	0.07*	0.07*	-0.03	0.06	-0.02	-0.15**	1		
m) Pearlin Mastery scale	-0.21**	0.14**	0.11**	-0.03	-0.07*	-0.04	-0.02	0.09**	-0.09*	0.07*	0.05	-0.46**	1	
n) Rosenberg Self-esteem scale	-0.11**	0.15**	0.02	0.01	-0.09**	-0.05*	-0.01	0.05	-0.03	0.07*	0.10**	-0.47**	0.47**	1

[a]: measured with Dutch Personality Questionnaire

*: Spearman's rho correlation coefficient $p < 0.05$

Figure 1 illustrates the associations between quartiles of personality characteristics and mean cortisol levels. As can be seen, none of the personality characteristics were significantly associated with cortisol levels; as the 95 % confidence intervals overlap each other. In line with this table 3 shows the results of the all-in linear regression analysis between personality characteristics, on a continuous scale, and log-transformed awakening and evening cortisol levels and diurnal variability of cortisol. No significant associations were observed between personality characterisitcs and cortisol levels. After adjustment for demographics the associations were weakened and further adjustment did not alter the associations greatly.

A significant interaction between age and neuroticism was found on evening cortisol level (B = -0.001; 95 % CI: -0.003 to 0.000; T = -2.50; df = 1139; p = 0.01), but not on awakening cortisol level (T = -0.61; df = 1139; p for interaction term = 0.52). When we stratified the sample in two age groups (<75 years vs. 75 years or older) higher neuroticism scores were significantly associated with higher evening cortisol levels in subjects younger than 75 years of age (fully adjusted model: B = 0.02; 95 % CI: 0.01 to 0.03; T = -2.67; df = 630;p =0.03), while no significant association between neuroticism and evening cortisol was observed in subjects 75 years or older (fully adjusted model: B = - 0.01; 95 % CI: -0.02 to 0.01; T= -1.32; df = 496; p = 0.19). The interaction terms between age and the other personality characteristics were not significant (p for interaction terms > 0.05).

When we dichotomized the personality characteristics at the 25% highest neuroticism and 25% lowest mastery and self-esteem scores, the results did not materially change (data not shown).

Table 3. Unstandardized coefficients from all-in linear regression analyses of the association between personality characteristics and awakening and evening cortisol levels (natural log-transformed) and diurnal variability (in %)

		Awakening cortisol*			Evening cortisol*		
		B (95 % CI)	T-statistic	p-value	B (95 % CI)	T-statistic	p-value
Neuroticism	Model 1[a]	-.002 (-.008 to .004)	-0.72	0.47	.008 (.000 to .015)	1.85	0.07
	Model 2[b]	-.002 (-.008 to .003)	-0.85	0.40	.007 (-.002 to .015)	1.56	0.12
	Model 3[c]	-.002 (-.008 to .003)	-0.81	0.42	.005 (-.003 to .014)	1.21	0.23
Mastery	Model 1	.006 (-.003 to .015)	1.40	0.16	-.011 (-.024 to .002)	-1.68	0.09
	Model 2	.008 (-.001 to .017)	1.74	0.08	-.005 (-.018 to .009)	-0.66	0.51
	Model 3	.008 (-.001 to .017)	1.70	0.09	-.004 (-.018 to .009)	-0.62	0.53
Self-esteem	Model 1	-.001 (-.013 to .013)	-0.31	0.76	-.012 (-.031 to .008)	-1.19	0.24
	Model 2	-.001 (-.013 to .013)	-0.00	0.99	-.006 (-.025 to .013)	-0.62	0.54
	Model 3	.000 (-.013 to .013)	0.02	0.99	-.004 (-.023 to .015)	-0.39	0.70

* As awakening and evening cortisol levels were natural log-transformed, the coefficients from linear regression analysis indicate that per one point increase on the personality questionnaires the cortisol levels change by 100*(regression coefficient) percent.

[a]: unadjusted (degrees of freedom = 1148); [b]: adjusted for age, sex, and level of education (degrees of freedom = 1145);

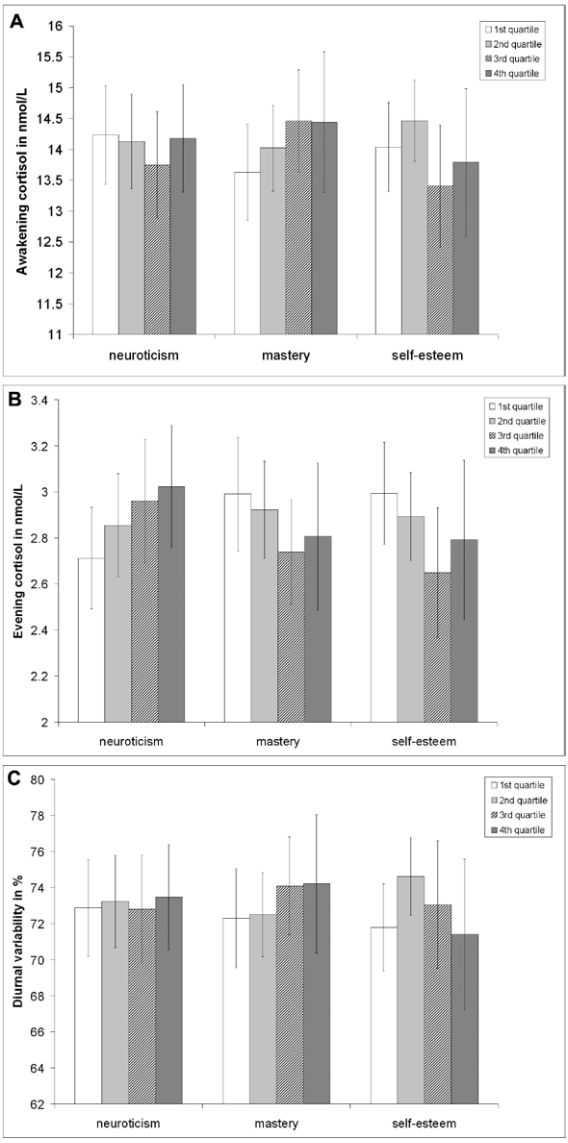
[c]: adjusted for age, sex, level of education, cardiovascular diseases, diabetes, hypertension, mean hours of sleep, BMI, smoking status and alcohol consumption (degrees of freedom = 1137)

Table 3 *continued*

	Diurnal variability			
		B (95 % CI)	T-statistic	p-value
Neuroticism	Model 1[a]	-.158 (-.424 to .107)	-1.17	0.24
	Model 2[b]	-.148 (-.417 to .120)	-1.08	0.28
	Model 3[c]	-.087 (-.357 to .183)	-0.63	0.53
Mastery	Model 1	.361 (-.060 to .781)	1.68	0.09
	Model 2	.307 (-.127 to .741)	1.39	0.17
	Model 3	.266 (-.165 to .697)	1.21	0.23
Self-esteem	Model 1	.209 (-.401 to .820)	0.67	0.50
	Model 2	.204 (-.416 to .825)	0.65	0.52
	Model 3	.118 (-.503 to .738)	0.37	0.71

[a]: unadjusted (degrees of freedom = 1148);
[b]: adjusted for age, sex, and level of education (degrees of freedom = 1145)
[c]: adjusted for age, sex, level of education, cardiovascular diseases, diabetes, hypertension, mean hours of sleep, BMI, smoking status and alcohol consumption (degrees of freedom = 1137)

Figure 1. Unadjusted cortisol levels per quartile of personality characteristics.



A: Awakening cortisol; B: Evening cortisol; C Diurnal variability (%).
Error bars represent 95 % confidence intervals.
1st qrt neuroticism: score <=1; 2nd qrt neuroticism: score 2 – 4; 3rd qrt neuroticism: score 5 – 8; 4th qrt neuroticism: score >= 9.
1st qrt mastery: score <=15; 2nd qrt mastery: score 16 – 18; 3rd qrt mastery: score 19 – 20; 4th qrt mastery: score >= 21.
1st qrt self-esteem: score <=14; 2nd qrt self-esteem: score 15 – 16; 3rd qrt self-esteem: score 17; 4th qrt self-esteem: score >= 18.

Discussion

In this large population-based study of elderly people, the personality characteristics mastery and self-esteem were not associated with HPA axis regulation as measured with salivary awakening and evening cortisol levels. However, we found some evidence that neuroticism was associated with higher evening cortisol levels in the younger old.

To our knowledge, this is the first study in which the associations of several personality characteristics with HPA axis regulation are examined in a large population-based cohort of older people. Preceding studies on the association between personality characteristics and HPA axis regulation have mostly been conducted in relatively young populations and reported inconsistent results. For instance, some studies reported no significant associations between neuroticism and HPA axis regulation [8, 10], whereas others found that high scores on neuroticism were related to elevated levels of cortisol [13, 14] or that high levels of neuroticism were associated with lower levels of cortisol [7, 9]. The inconsistent results may be explained by the type of cortisol sample used (e.g. blood; urine; saliva), use of basal cortisol levels or cortisol responses to psychosocial stress or CRH/dexamethasone tests and differences in study population (e.g. young; old; healthy; depressed). Because of these methodological differences it is difficult to compare our findings on neuroticism and HPA axis regulation with previous studies. Also, none of the studies on personality characteristics and HPA axis regulation included evening cortisol levels. Furthermore, the personality characteristic mastery has not been investigated before with respect to HPA axis regulation, although Pruessner et al. did include the construct internal control, but investigated it in combination with self-esteem [12, 17, 18].

Only three small studies have been performed in older persons, in which the association between self-esteem and HPA axis regulation was examined [17-19]. All reported a negative association between self-esteem and basal cortisol levels or cortisol responses to stress. In contrast with these studies we did not find a significant association between self-esteem and cortisol levels. It is possible that the mean age of our cohort was too high to find an association.

A strength of our study is that salivary samples were collected after awakening and late in the evening. Preceding studies examining the association between personality characteristics and HPA axis regulation did not include evening cortisol samples. Combining awakening and evening cortisol samples made it possible to study the diurnal variability of cortisol. Another strength of this study is that the study sample consisted of a large population-based cohort of older people, and that we had information on a large number of covariates, for which the associations could be adjusted.

A limitation of our study is that the study sample consisted of persons who were still able to participate in the fourth follow-up of LASA and who were willing to collect saliva for cortisol measures. Consequently, the findings may not be representative for

other populations of older persons. Also, since the participants were asked to collect saliva within 30 minutes after awakening, our measure confounds a true awakening levels with the known rise at 30-45 minutes post awakening. Therefore we are unable to trust that these results represent what other papers report as awakening level or a waking rise. This could have contributed to an overall non-significant association between personality indicators and cortisol levels. Additionally, it has also been suggested that cortisol samples should be collected on several consecutive days, which we did not do [34]. Cortisol levels are highly variable within an individual and sampling on one day may lead to diluted estimates. Another limitation is that we had no information on hours of sleep, the time of awakening and problems with sleeping. These are all factors that may influence HPA axis regulation and may be associated with personality characteristics. We adjusted for the mean hours of sleep, however, and this did not change the results.

Furthermore, in large cohort studies like ours loss to follow-up is a common problem, which can lead to selection bias. To overcome this problem we imputed missing data using single imputation. A simulation study showed that single imputation gives an unbiased estimate of the association [26]. However, since the single imputation procedure gives a smaller standard error it can lead to overly liberal significance tests.

In summary, the findings of this large population-based study of older persons suggest that the personality characteristics mastery and self-esteem are not associated with HPA axis regulation as measured with salivary awakening and evening cortisol. However, high neuroticism may be associated with elevated levels of evening cortisol in the younger old, but not in the older old.

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2.2 Early and late life events and salivary cortisol in older persons

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Abstract

Background

It has been hypothesized that stressful life events are associated with changes in (HPA) axis regulation, which increases the susceptibility to psychiatric disorders. We investigated the association of early and late life events with HPA-axis regulation in older persons.

Methods

Within the Longitudinal Aging Study Amsterdam (LASA) 1,055 participants (47% male), aged 63-93 years, collected saliva within 30 minutes after waking and late in the evening. Early and late life events were assessed during a home interview. The associations between life events and cortisol levels were examined using linear regression and ANCOVA with adjustments for demographics, cardiovascular risk factors and depressive symptoms.

Results

Within our study sample, the median morning and evening cortisol levels were 15.0 nmol/L (10-90%:7.4 – 27.0 nmol/L) and 2.8 nmol/L (10-90%:1.5 – 6.3 nmol/L) respectively. Persons who reported early life events showed lower levels of natural log-transformed morning cortisol (B=-0.10; 95%CI: -0.17 to -0.04) and flattened diurnal variability of cortisol (B=-1.06; 95%CI -2.05 to -0.08). Persons who reported two or more late life events showed higher levels of natural log-transformed morning cortisol (B=0.10; 95%CI:0.02 to 0.18) and higher diurnal variability (B=1.19;95%CI:0.05 to 2.33). No associations were found with evening cortisol.

Conclusion

The results of this large population-based study of older persons suggest a differential association of early and late life events with HPA axis regulation; early life events were associated with a relative hypo-secretion of morning cortisol and flattened diurnal variability, while late life events were associated with elevated secretion of morning cortisol and high diurnal variability of cortisol.

1. Introduction

In a large variety of studies it has been observed that the experience of stressful life events constitutes a major risk for the development and persistence of psychiatric disorders [1-3]. Stressful life events and psychiatric disorders such as depression may be associated with changes in glucocorticoid levels [4-6]. It has been hypothesized that stress leads to long-term changes in hypothalamic-pituitary-adrenal (HPA) axis regulation [1;7], which, in turn, makes an individual more prone to psychiatric disorders [8]. Furthermore, it has been hypothesized that particularly severe stress during childhood leads to chronic alterations in HPA axis regulation [9;10].

Studies on the relation between adverse events during childhood and HPA axis regulation resulted in inconsistent findings: some studies reported elevated levels of basal cortisol [11;12] and hyper-reactivity to new stressors [13], while others reported lower basal cortisol levels [14;15] and hypo-reactivity to new stressors [16] after early adverse events.

With respect to life events later in life, conflicting results have been observed as well. Several studies found that adverse events later in life were associated with lower levels of salivary morning cortisol and 24-hour plasma cortisol, particularly in persons with PTSD [17;18], but elevated levels of salivary and 24-hour urine cortisol have been reported following adverse events later in life as well in persons with [19;20] and without PTSD [21].

Methodological differences, including variation in study population (children, adolescents or adults), participants with or without psychopathology (e.g. depression or post-traumatic stress disorder), nature of adverse event (neglect, abuse, maltreatment), cortisol measurements (e.g. basal cortisol versus cortisol reactivity tests) and type and onset of stressful events may explain these conflicting results [5;22;23].

Since HPA axis regulation is also affected by aging, it has been suggested that older people show a different stress-reactivity than younger adults [24]. Older persons experience many life events, predominantly of negative valence, and are thus exposed to many stressors [25]. However, most of the previous studies were conducted in relatively small samples of children or younger adults (age < 65 years), and less is known about the association between life events and cortisol in older adults.

The present study assessed the association between early and late life events and HPA axis regulation, using basal salivary cortisol levels, collected within 30 minutes after awakening and in the evening, in an older community-based population.

2. Methods

2.1 Sample

Data were derived from the Longitudinal Aging Study Amsterdam (LASA). LASA is a population-based prospective cohort study among persons initially aged 55–85 years. Details of this cohort study have been described previously [26]. For the present study, data from the baseline interview of LASA (T1) were used for data on early life events, whereas all other variables were assessed at the third follow-up of 2001-2002 (T4), when 1,691 subjects participated. Of the 1,416 persons who were lost to follow-up, 1,050 (33.8% of the T1 sample) had died, 112 (3.6%) indicated by self-report or proxy that they were too ill or cognitively impaired to be interviewed, 222 (7.1%) indicated that they were no longer interested in participating in the study, and 32 (1.0%) could not be contacted.

During the third follow-up (T4), from the 1.619 available respondents, 1,474 underwent a face-to-face interview and 1,307 respondents underwent a medical interview, during which additional information was gathered, including collection of saliva samples. Interviews were conducted at the homes of the respondents by well-trained and intensively supervised interviewers. All interviews were tape-recorded to control the quality of the data.

2.2 Measures

Cortisol measurement

Saliva samples were collected with the "Salivette" device from Sarstedt, in Nümbrecht, Germany. Material for collection of saliva and detailed instructions were sent home to the respondents, and the samples were kept in the refrigerator until collected by the interviewer the next day. Respondents were instructed to collect saliva in the morning within 30 minutes after waking and in the evening just before going to bed. In order to prevent contamination with blood, respondents were instructed to rinse their mouth and wait ten minutes before starting to chew the cotton ball and not to eat or brush their teeth before.

Cortisol levels (nmol/L) were determined using radio immunoassay coated tubes (Spectria Orion Diagnostics, Finland). The detection limit was 1.5 nmol/L. The intra-assay coefficient of variation was 7% for cortisol levels around 30 nmol/L and 19% for cortisol levels around 1.3 nmol/L, whereas corresponding inter-assay coefficients of variation were 5% and 19% [27]. Morning and evening cortisol levels had a skewed distribution. To obtain normal distribution of cortisol levels natural log transformation was performed. Diurnal variability of cortisol was calculated by subtracting the evening levels from the morning levels.

Life events

Late life events were measured with a structured questionnaire during the face-to-face interview and were assessed retrospectively during T4. Respondents were asked whether the following events had occurred in the three-year time interval prior to the interview: widowhood, divorce, severe illness of partner, death of a relative, death of a child, relocation, severe conflicts, victim of a crime, or financial problems. The events were adapted from the life event inventory developed by Tennant and Andrews [28], with the aim of assessing the stress of life events. The significance of life events is scaled in two conceptually different ways. The first is based on the magnitude of the change produced by the event. The second is based on the undesirable or distressing quality of the events. The items used in the LASA data collection were selected on the following criteria: the event is likely to occur relatively frequently in the population (e.g., having a baby is unlikely), and the event scores relatively high on the distress and life changes scalings [29]. In the analyses, the number of late life events was categorized into three more or less equally sized categories: no late life events, one late life event and two or more (with a maximum of 5) late life events.

Early life events were assessed by means of an open-ended question whether the participant had experienced any significant life events before the age of 18. The reported life events were categorized into war experiences; death of a parent or important other; divorce of parents; sexual abuse; severe problems at home; poverty or unemployment; and physical illness. In the analyses, respondents were categorized in having or not having experienced a significant life event during childhood.

Additionally, based on the life events measures a new variable was created which combined early and late life events, with the following four categories: 1) no early or late life events; 2) only early life events; 3) only late life events; and 4) both early and late life events.

Other variables

Age, sex and level of education were recorded during the first wave of LASA (T1). Educational level was recorded in seven categories ranging from not more than 6 years of education to university education. Other covariates were assessed at T4. In addition, the presence of diabetes, history of cardiovascular diseases (atherosclerosis, heart disease and cerebrovascular accidents) and medication use were assessed based on self-report [30]. Blood pressure was measured twice, and the average of two measurements was calculated. Hypertension was defined as a diastolic tension >90 mmHg or a systolic tension above 140 mmHg or usage of anti-hypertensive medication. Body Mass Index (BMI) was calculated from measured weight and height and expressed as kg/m^2. Furthermore, alcohol consumption, smoking status and hours of sleep were assessed. Alcohol consumption was expressed as number of drinks per week and was categorized in three groups (no alcohol use; on average <14 drinks weekly; ^{3}14 drinks weekly). Smoking habits were categorized into current and non-

current smoker. Respondents were asked to determine the average number of hours of sleep per night. Depression was measured using a Dutch version of the Center for Epidemiologic Studies Depression (CES-D) questionnaire [31;32].

2.3 Study sample

Of the 1,307 respondents participating in the medical interview at T4, 1,184 (90.6%) respondents collected saliva for cortisol measurements. Thirty-four respondents (2.7%) used corticosteroids and were excluded from the study sample. Four samples of morning levels and 92 samples of evening values were below the detection limit (1.5 nmol/L). We imputed these missing values with random values between 0 and 1.5. Furthermore, morning and evening cortisol levels above 100 nmol/L were excluded, because these extreme levels were considered to be due to measurement error (10 morning samples and 5 evening samples; 1.3 % of all cortisol samples) and also if the evening level was more than 5 nmol/L higher than the wakening level (22 samples). The cut-off of 100 nmol/L corresponds to a cut-off score of 4 SD above the mean morning cortisol level [27]. We recoded these values to missing values. Missing values were also present on covariates in 48 respondents, leaving 1,049 participants for morning cortisol levels; 1,055 participants for evening cortisol levels and 1,041 participants for diurnal variability of cortisol for the data-analysis.

Compared to the respondents of whom no complete data was available (n = 636), the study sample was four years younger was more often male, had fewer chronic illnesses, and had a higher score on the Mini Mental State Examination ($p < 0.05$). They did not differ with respect to level of education, alcohol consumption, smoking habits, or average hours of sleep per night.

2.4 Data-analysis

Linear regression analyses and analysis of covariance (ANCOVA) were used to estimate the cross-sectional associations of childhood adversity and number of life events with morning and evening levels of cortisol and diurnal variability of cortisol. In separate models the diurnal variability of cortisol and the natural log transformed levels of morning cortisol and evening cortisol were entered as the dependent variable. We first examined the single effects of early life events (yes vs. no) and number of late life events (0,1, 2 or more) by entering them in separate linear regression models as independent variable and then created an ANCOVA model for the combined life events variable (no reported life events; only early life events; only late life events; early and late life events). Firstly, models were adjusted for age and sex. Secondly, models were also adjusted for educational level, smoking, alcohol consumption, BMI, hypertension, diabetes, cardiovascular diseases and average number of hours of sleep. And thirdly, additional adjustments were made for depressive symptoms. We selected these covariates a priori, based on the literature [33-36] and tested whether they also fulfilled the criteria for confounding within our data by adding each confounder to the

linear regression models between life events and cortisol measures. If the coefficient changed with 10 % or more we added the confounder to the model.

Finally, because some studies suggest different cortisol levels after psychosocial stress in men and women [16;24], interaction terms between sex and life event variables were added to the fully adjusted model.

3. Results

In the total study sample the median morning cortisol level was 15.0 nmol/L (10-90%: 7.4 – 27.0 nmol/L), the median evening cortisol level was 2.8 nmol/L (10-90%: 1.5 – 6.3 nmol/L) and mean diurnal variability of cortisol was 12.4 (SD 7.4). Table 1 presents the baseline characteristics of the study sample (n=1,055) per life event category. As can be seen, persons who reported only early life events were slightly younger, had a higher educational level and had less often diabetes than the other categories. Persons who reported only late life events more often had a history of cardiovascular diseases.

Table 1 baseline characteristics per life event category

	No reported life events	Only early life events	Only late life events	Early and late life events
N	256	87	465	247
Male (%)	50	52	46	45
Age, mean (sd)	75.0 (6.8)	72.8 (6.7)	75.2 (6.9)	73.6 (6.9)
Level of education (0-9), mean (sd) [1]	3.6 (1.9)	4.2 (2.1)	3.6 (1.9)	3.9 (2.2)
BMI, mean (sd)	27.3 (4.3)	27.2 (4.6)	27.4 (4.1)	27.6 (4.2)
Cardiovascular diseases (%)	30	29	37	28
Hypertension (%)	31	30	32	28
Diabetes (%)	6	2	7	8
Current smoker (%)	18	17	13	17
Alcohol consumption (%)				
- no consumption	22	15	17	20
- < 14 drinks/week	61	54	61	56
- ≥ 14 drinks/week	17	31	22	24
Hours of sleep, mean (sd)	7.3 (1.4)	7.3 (1.5)	7.4 (1.3)	7.2 (1.4)
CES-D[2], median (0-90 %)	6.0 (1.0 – 18.0)	8.0 (0.0 - 19.6)	7.5 (1.0 -19.0)	9.0 (2.5 – 21.0)

[1] Level of education ranges from elementary education or less <= 6 years of education to college education and higher vocational education.
[2] Center for Epidemiologic Studies Depression scale (0 – 30).

Table 2 presents the frequency distributions of the early and late life events. War experiences, severe problems at home and death of parents were the most often reported early life events. The most often reported late life events were death of a relative, severe illness of a relative and severe illness of partner.

The results from the linear regression analyses are shown in table 3. Persons who reported early life events had significantly lower morning cortisol and flattened diurnal variability of cortisol, compared to persons who reported no early life events (model 2). No significant associations were found for evening cortisol. After additional adjustment for depressive symptoms (model 3) the associations with morning and evening cortisol did not materially change. However the association with diurnal variability of cortisol slightly attenuated ($p = 0.07$).

Persons who reported two or more late life events in the past 3 years had significantly higher morning cortisol compared to persons who reported one late life event or no late life events and two or more late life events were borderline significantly associated with higher diurnal variability of cortisol ($p = 0.06$) (model 2). No significant associations were found on evening cortisol. Additionally, the associations with morning and evening cortisol did not materially change after adjustment for depressive symptoms in the third model, but the association with diurnal variability of cortisol changed from borderline significant to significant.

Table 2. Frequency distribution of type of childhood adversity and recent life events

Type of life event	N (% of reported events)
Early life events (total reported = 369):	
War experiences	115 (31.1)
Death of parent(s)	45 (12.2)
Death of important other	17 (4.6)
Parents divorced	8 (2.2)
Sexual abuse	8 (2.2)
Physical problems	16 (4.3)
Severe problems at home	80 (21.7)
Poverty or unemployment	4 (1.1)
No answer	5 (1.4)
Not classifiable	71 (19.2)
Late life events (total reported = 1,342)	
Widowhood	58 (4.3)
Divorce	0 (0)
Severe illness partner	132 (9.8)
Death of relative	378 (28.2)
Death of child	114 (8.5)
Severe illness relative	445 (33.2)
Relocation	105 (7.8)
Conflict with others	91 (6.8)
Victim of crime	11 (0.8)
Financial problems	8 (0.6)

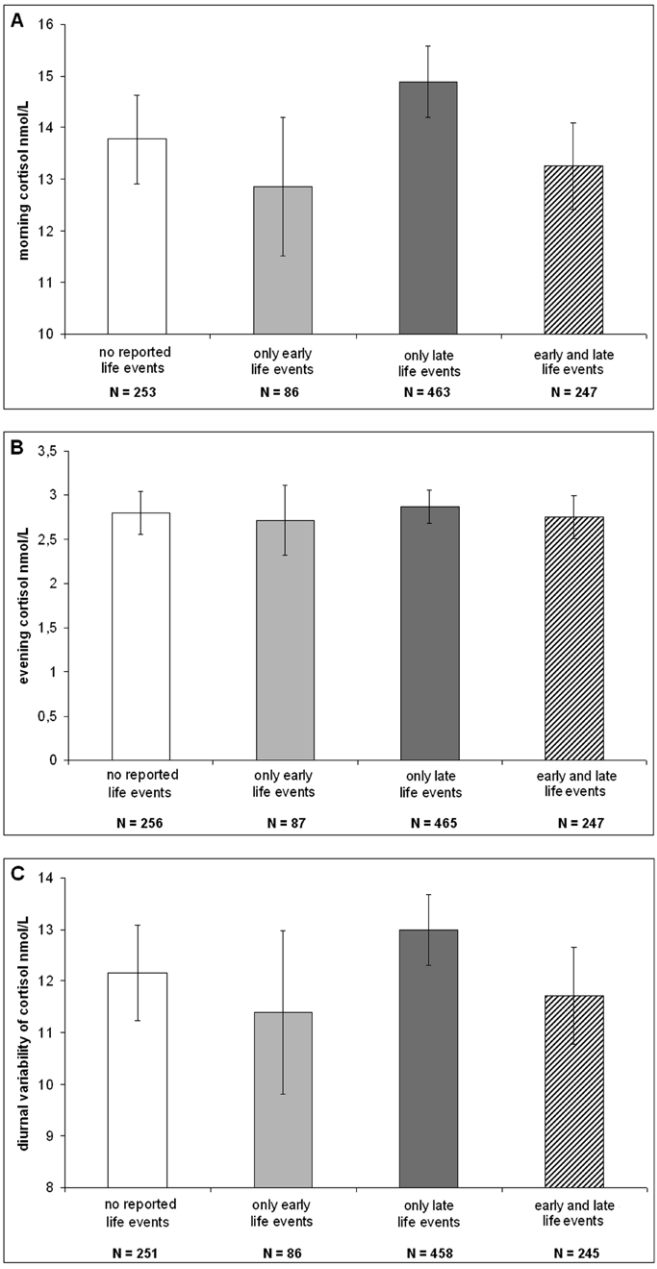
Table 3. Results of the regression analyses of the associations between childhood adversity, life events and natural log-transformed morning and evening cortisol levels and diurnal variability of cortisol

		LN morning cortisol	**LN evening cortisol**	**Diurnal variability in nmol/L**
		B (95 % CI)	B (95 % CI)	B (95 % CI)
Early life events		Ref	Ref	Ref
- No early life events	crude	-0.09 (-0.16 to -0.03)*	-0.06 (-0.16 to 0.03)	-0.96 (-1.93 to 0.01)
	Model I	-0.09 (-0.16 to -0.02)*	-0.05 (-0.14 to 0.05)	-1.02 (-2.00 to -0.04)*
- Early life events	Model II	-0.10 (-0.17 to -0.04)*	-0.04 (-0.13 to 0.06)	-1.06 (-2.05 to -0.08)*
	Model III	-0.09 (-0.16 to -0.02)*	-0.04 (-0.13 to 0.06)	-0.93 (-1.92 to 0.06)
Late life events		Ref	Ref	Ref
- No late life events	crude	0.01 (-0.06 to 0.09)	0.00 (-0.11 to 0.11)	0.17 (-0.93 to 1.27)
	Model I	0.02 (-0.06 to 0.09)	0.02 (-0.09 to 0.12)	0.15 (-0.94 to 1.25)
- One late life event	Model II	0.01 (-0.06 to 0.09)	0.01 (-0.10 to 0.12)	0.14 (-0.96 to 1.24)
	Model III	0.02 (-0.06 to 0.09)	0.01 (-0.10 to 0.12)	0.18 (-0.92 to 1.28)
- Two or more late life events	crude	0.10 (0.03 to 0.18)*	0.05 (-0.06 to 0.16)	1.11 (-0.02 to 2.24)
	Model I	0.09 (0.02 to 0.17)*	0.03 (-0.09 to 0.12)	1.10 (-0.04 to 2.23)
	Model II	0.10 (0.02 to 0.18)*	0.03 (-0.08 to 0.14)	1.08 (-0.06 to 2.22)
	Model III	0.11 (0.03 to 0.19)*	0.03 (-0.08 to 0.14)	1.19 (0.05 to 2.33)*

Model I: adjusted for age and sex
Model II: additional adjustments for educational level, BMI, alcohol use, mean hours of sleep, diabetes, cardiovascular disease, hypertension and current smoking.
Model III: Model II + adjustments for depressive symptoms. * significant association $p < 0.05$

Figure 1a-1c shows the adjusted mean cortisol levels for the combined life event categories (no reported life events; only early life events; only late life events; and both early and late life events). Mean cortisol levels were transformed back to linear scale. Overall, there was a significant relation between life event categories and morning cortisol ($F_{(3,1046)} = 3.82$; $p = 0.01$) (figure 1a) . There was no significant relation between life event categories and evening cortisol ($F_{(3,1052)} = 0.24$; $p = 0.87$) (figure 1b). A borderline significant relation was found between life event categories and diurnal variability of cortisol ($F_{(3,1042)} = 2.17$; $p = 0.09$) (figure 1c). After additional adjustment for depressive symptoms the relations between life events and morning and evening cortisol did not materially change, but the relation with diurnal variability was no longer significant ($F_{(3,1042)} = 1.89$; $p = 0.13$).

Figure 1. Adjusted means of morning (A), evening (B) cortisol levels and diurnal variability of cortisol (C) (± 95 % confidence interval) per life event category



Mean cortisol levels were adjusted for age, sex, educational level, BMI, alcohol use, mean hours of sleep, diabetes, cardiovascular disease, hypertension and current smoking.

No significant interaction was found between early and late life events for morning or evening cortisol levels or diurnal variability of cortisol. Also, no significant interactions were found between sex and early or late life events for morning or evening cortisol levels or diurnal variability of cortisol ($p > 0.05$; data not shown).

4. Discussion

In this large community-based sample of older persons we observed that life events experienced later in life were associated with higher morning levels of cortisol, whereas life events experienced early in life were associated with lower morning levels of cortisol. Also, early life events were associated with flattened diurnal variability of cortisol, whereas late life events were associated with higher diurnal variability of cortisol.

To our knowledge, this is the first study to investigate the association of early and late life events with HPA axis regulation in older persons. A major strength of our study is the large population-based sample and the extensive measurements of a large number of covariates, for which analyses could be adjusted. Furthermore, the response rate for saliva collection was very high in our study sample, which led to limited selection bias.

A limitation of the study is the cross-sectional design. As a result, we cannot discern cause from consequence. We therefore do not know if cortisol levels changed after the experience of life events or that changes in cortisol levels already existed prior to the experience of life events. Since we assessed life events retrospectively we assume that life events were experienced prior to the observed cortisol levels.

Another limitation of our study is that we do not know exactly at which time the saliva samples were taken, as the participants were asked to collect saliva within 30 minutes after awakening instead of directly at awakening or 15 or 30 minutes after awakening. Cortisol follows a specific circadian rhythm, with peak levels 30 minutes after awakening when cortisol levels increase with 50-60 % [37] and a nadir at night. Consequently, it is unclear whether the morning samples in our study reflect the awakening level, the peak at 30 minutes after awakening, or any time within this 30 minute interval. Although this will have lead to measurement errors, we do not think that this error was systematic, as it seems unlikely that persons who reported late life events collected their morning samples systematically later than persons who reported early life events. More likely, this sampling method resulted in non-differential misclassification and may have diluted our estimates and it is therefore recommended that studies sample cortisol with greater attention to it's circadian rhythm. Additionally, it is recommended to collect cortisol samples on two consecutive days [38], because cortisol levels are rather variable within individuals. However, our large sample size may have largely compensated for the possible variability in cortisol measures.

It has been proposed that life events experienced in childhood (younger than 12 years of age) have a stronger association with HPA axis regulation than life events experienced during adolescence (12 to 18 years of age); as it has been suggested that particularly life events during early childhood affect the development of the HPA axis [39]. In our study, we asked respondents about life events before the age of 18 years and thus it is possible that we underestimated the association between early life events and HPA axis regulation. On the other hand, this age cut-off has been used in many other studies [40;41] and has been shown to be a reliable and valid cut-off for measuring childhood adversity [42;43].

Additionally, the early life events were assessed using an open-ended question. Therefore it is possible that we missed certain important childhood events. It is possible that some underreporting has occurred due to unwillingness to report embarrassing events or painful memories. Underreporting may also have occurred because of some memory failures. However, in our study sample, the level of global cognitive functioning, as measured with the Mini-Mental State Examination [44], was well within normal ranges, therefore recall failure due to cognitive impairment will probably be limited.

In spite of the shortcomings, our results suggest a differential association of early and late life events with HPA axis functioning. As first noted by Selye [45], stressors cause an initial hyper-activation of the HPA axis. However, there is increasing evidence that exposure to severe stressors or chronic stress can also lead to relative hypo-activation of the HPA axis [9;10;22;46]. For instance, in two recent reviews it has been suggested that hyper-secretion of cortisol could be a marker for acute stress, while hypo-secretion is more likely to be a consequence of chronic stress, but could also be related to longer passing of time since the onset of a stressor [22;23]. The exact mechanism by which this occurs is still unclear. A possible mechanism may be that long-lasting excessive secretion of cortisol leads to negative feedback inhibition of the pituitary to the adrenal cortex [9;10].

Additionally, it is believed that severe stressors early in life can set the responsiveness of the HPA axis. For instance, in rodents it has been found that after neonatal handling, the HPA axis underreacts to stressors later in life [9]. In humans it has also been found that people who experienced adverse childhood events showed blunted cortisol levels after a psychosocial stress task or a corticotrophin-releasing factor stimulation test [13;16]. However, in our study, persons who reported both early and late life events showed no significant alterations on basal salivary cortisol levels compared to persons who reported no life events or only early life events. Probably, the differential associations of late life events with higher levels of morning cortisol and diurnal variability and early life events with lower morning cortisol levels and flattened diurnal variability cancelled out an interactive effect between early and late life events on HPA axis regulation.

We did not observe any significant associations between life events and

evening cortisol levels; as a result the differences in diurnal variability are largely due to the differences in morning cortisol levels. Previous studies on HPA axis regulation and adverse life events [14;47] also reported that differences in diurnal variability could be largely explained by differences in morning cortisol and less by evening cortisol levels, although one study reported that physical and psychological abuse in women were associated with higher levels of evening cortisol and not with differences in morning cortisol [21]. In our study, the intra- and inter-assay coefficients of variations of the laboratory measurements of cortisol were relatively high for low levels of cortisol. This may have affected the reliability of the evening levels and may have reduced the possibility of finding significant associations with evening cortisol.

Besides stress, HPA axis regulation can be affected by numerous other factors, one of which is aging [24;48]. Since we are among the first to study the relation between life events and HPA axis regulation in older persons more research is necessary in order to investigate to which extent the reactivity of the HPA axis is altered by life events in old age. However, our results in older persons are comparable to several previous studies in younger adults, which observed similar differential associations of early and late life stress with cortisol levels [5;49].

Several lines of evidence suggest that life events and HPA axis regulation are both associated with depression [1;7]. Possibly, the experience of life events leads to chronic changes in HPA axis regulation, which could increase the risk for psychiatric disorders, like depression [8]. To study the effect of depression on the association between life events and HPA axis regulation we adjusted for depressive symptoms in a separate model. Adjustments for depressive symptoms hardly altered the associations between life events and salivary cortisol levels, which suggests that life events are associated with HPA axis regulation independently of depression. Two previous studies already showed that life events and cortisol levels are independently related to onset of depression during follow-up [50;51], whereas one cross-sectional study observed that in depressed women psychosocial stressors were related to elevated evening cortisol, but not in non-depressed women [52].

It has also frequently been reported that HPA axis regulation is affected by sex [16;24]. In these stress induction studies it has been observed that the cortisol response to challenge is higher in older women than in older men. In our study we did not find that the association of life events with cortisol levels was different in men and women. However, we investigated basal cortisol levels in relation to life events and not the stress-reactivity of the HPA axis. Therefore our data may not be comparable to studies in which stress-reactivity was examined.

In conclusion, the results of this large population-based study of older persons suggest a differential association of early and late life events with HPA axis regulation; early life events were associated with a relative hypo-secretion of morning cortisol and flattened diurnal variability, while late life events were associated with elevated secretion of morning cortisol and high diurnal variability of cortisol

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Chapter 3

Stress, HPA-axis and cognition

3

3.1 Associations between serum cortisol and cognitive decline in older persons

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Abstract

Objective
To investigate whether serum cortisol levels are associated with cognitive performance and cognitive decline in elderly persons, and whether this association differs by age, sex, and depression status.

Design
Data from the Longitudinal Aging Study Amsterdam (LASA), with repeated measurements of cognitive performance after 3 and 6 years.
Participants: 1,154 persons, aged 65-88 years.

Measurements
Serum concentrations of total cortisol (CRT) and Corticosteroid Binding Globulin (CBG) were measured at baseline, and from these Free Cortisol Index (CRT/CBG) was computed. At baseline, and 3 and 6 years of follow-up, global cognitive functioning, verbal memory performance, and speed of information processing were assessed.

Results
After adjustment for demographics, health and lifestyle variables, a significant association between high levels of free cortisol and poorer performance on verbal learning (B= -0.32; 95%CI: -0.64 to -0.01) was found in both women and men. Additional adjustment for depression did not change this association. In women, but not in men, high levels of free cortisol (B=-0.85; 95 % CI: -1.40;-0.31) were associated with slower speed of information processing. The associations between cortisol and cognitive performance were the same for the younger and the older old, and for depressed and non-depressed persons. Higher levels of cortisol were not associated with cognitive decline over a period of 6-years.

Conclusion
Our study provides further evidence that high levels of cortisol measured during the day are associated with lower memory function and speed of information processing, but not with decline in cognitive functioning over 6 years of time.

1. Introduction

There is growing evidence that the stress hormone cortisol is associated with cognitive impairment in older persons.[1,2] Cortisol is one of the products of the hypothalamic-pituitary-adrenal (HPA) axis, which facilitates the fight-or-flight response. Although this stress response is useful for the adequate activation of functions needed to re-establish homeostasis after a stressful situation, inadequate or prolonged responses may increase the risk of disease.[3,4] Cortisol may negatively influence cognitive performance, because it can cross the blood-brain barrier and bind to receptors localized in the hippocampus, amygdala and frontal lobes, known to be involved in learning and memory.[2] In addition, acute administration of synthetic glucocorticoids has been shown to impair memory function in humans,[5] and also animal studies found support for adverse effect of cortisol on cognitive performance.[6]

The majority of prior studies investigating the association between cortisol levels and cognitive decline were performed in younger adults. Fewer studies have been conducted in older persons, despite the fact that detrimental effects on cognition may be of special interest to older persons because cognitive decline in older persons is highly prevalent and may partly be reversible.[7-9] Little is known about conditions that cause (temporary) cognitive decline in older persons. Cortisol may be such a condition, since in younger adults it has been shown that cognitive impairments reversed when cortisol levels decreased to normal.[10,11] In addition, several observational studies found an association between high levels of cortisol and cognitive impairment[12-18] and cognitive decline.[16,19]

Although results of prior aging studies consistently indicate an adverse effect of high cortisol on cognitive functioning, some issues regarding the effect of cortisol on cognitive decline warrant further research. First, reactivity of the stress system has shown to be associated with aging[1,20, 21, 27, 28] and sex.[21,22] For instance, older age has found to be associated with higher levels of cortisol[21]. However, in older persons hypo-reactivity of the stress system has also been found, leading to low levels of cortisol.[27,28] According to Lupien et al,[29] low levels of cortisol may also influence cognitive function. They showed in an experimental study that the absence of circulating cortisol levels are as detrimental for human memory function as is an increase in cortisol. Thus far, there are no studies that have investigated a possible U-shaped relation between cortisol and cognitive decline. With respect to differences between men and women in reactivity of stress system the findings are confusing. Larger free cortisol responses have been shown in older men than in older women[22], but the opposite has also been found[21]. Therefore, it is important to further investigate whether the association between cortisol and cognitive decline is similar for the younger and the older old, and for men and women. Second, late-life depression is also often associated with cognitive decline,[23, 24] but also with high levels of cortisol.[25-28] Therefore, it is of interest to investigate whether the association between cortisol and cognitive decline is

influenced by the presence of depressive symptoms. Third, previous studies suffered from small sample sizes[21,30] and/or were not always able to control for possible confounders, especially lifestyle variables, although they may have an important impact on the association between cortisol and cognitive decline.
In the present study, we will investigate whether levels of cortisol, measured in serum, are associated with cognitive performance and increased risk for cognitive decline, and whether this association differs by age, sex, and depression status. Data are used from the Longitudinal Aging Study Amsterdam, which also provides information on somatic health, lifestyle characteristics and depressive symptoms, that may confound the association under study.

2. Methods

Study sample

The Longitudinal Aging Study Amsterdam (LASA) is a population-based study of persons in the Netherlands initially aged 55 to 85 years.[31] LASA is based on a random sample, stratified for age and sex, drawn from the population registries of 11 municipalities. In 1992/1993, 3,107 subjects were enrolled in the baseline LASA interview (T1), with a response rate of 81.7%. The first follow-up measurement took place in 1995/1996 (T2; N=2,545), the second in 1998/1999 (T3; N=2,076) and the third in 2001/2002 (T4; n=1,691). Loss to follow-up was due to mortality (33.8%), serious illness or cognitive impairment (3.6%), and refusal (7.1%); an additional 1% could not be contacted. Data were collected during a general face-to-face interview and a medical interview, during which additional data were gathered (e.g. weight, vision test, cognitive tests). The study sample consisted of subjects who were born in 1930 or before (aged 65 years and older) and who participated in the medical interview of the first follow-up (T2; n=1,509). After face-to-face interview and a medical interview at home, participants were invited to health care centers near their homes, where blood samples were obtained. Blood samples from which cortisol could be determined were available for 74% of the participants (n=1,273). Compared to the 1,509 eligible respondents, those who did not participate in the blood drawing procedure were older, had lower education, had higher depression scores, and had lower scores on all cognitive tests (all $p<0.05$). Respondents using either oral glucocorticoids (n=26), sex-steroids (n=21) or mineral corticoids (n=11) were excluded in the present analysis. Finally, 61 respondents were excluded because of probable dementia (persistent cognitive decline of more than two SD below the mean over three measurements[32]) or missing values on cognition measures, resulting in a sample of 1,154 respondents.

Measurements

Cognitive function was assessed at baseline and two follow-up measurements with a set of widely used cognitive tests that are sensitive to decline with aging, and associated with different brain areas. The tests capture the following domains: general cognitive performance, episodic memory and information processing speed.

General cognitive performance was measured with the Mini-Mental State Examination (MMSE),[33] a frequently used screening instrument for global cognitive dysfunction.

Episodic memory was measured by means of the adapted Dutch version of the Auditory Verbal Learning Test (AVLT).[34] This test consists of 15 words, which had to be learned during three trials. The total number of words the respondent had learned during the three presentations is the *learning* score, which ranges from 0-45. The ratio of the maximum number of words at one of the trials at immediate recall and the delayed recall is defined as the *retention* score. This reflects the percentage of words which the respondent still remembered after a distraction period relative to the learning phase.

Information processing speed was measured by means of an adjusted version of the Coding Task.[35] In this task two rows of paired alphabetical letters were shown. The respondent was then offered just one alphabetical letter and had to vocally mention the missing paired second alphabetical letter. This was done in three trials of one minute. The maximum score of one of the three trials is included in the present study.

Serum cortisol, corticosteroid binding globulin and the free cortisol index

Blood samples were collected in the morning before 10.00 am, participants were allowed to take tea and toast before, but no dairy products. Samples were centrifuged and serum was stored at –70 °C until processing in 2002/2003. Serum cortisol levels (nmol/L) were determined using a commercially available competitive immunoassay ACS: Centauer developed by Bayer Diagnostcs in The Netherlands. Cortisol binding globuline (CBG) levels in mg/L were determined using a radio immunoassay from Medgenix Diagnostics in Belgium. The lower limit for accurate detection of cortisol was 30 nmol/L, the inter-assay and intra assay coefficient of variation (CV) were below 8% and 3% respectively. The lower limit for accurate detection of CBG concentrations was 11 mg/L and inter- and intra-assay CVs were both below 8%. In none of the samples the concentrations of cortisol and CBG fell below lower detection limits.[25] Since 90% of cortisol is bound to CBG and only the free fraction is considered to be biologically active we computed a free cortisol index (FCI) as total cortisol/CBG ratio.[36]

Covariates

Baseline sociodemographic variables included age, sex and level of education. Level of education was classified into 9 categories, ranging from 1 = "elementary school not completed" to 9 = "university education". Information on hypertension, cardiovascular diseases, diabetes, and stroke was obtained by self-report[35]. Lifestyle variables included Body Mass Index (BMI), current alcohol intake (no alcohol, average <14 drinks

a week, ≥ 14 drinks a week) and smoking (no, current smoking). BMI was calculated as [weight (kg)/height (m)^2]. Depressive symptoms were measured by means of the Dutch translation of the Center for Epidemiologic Studies Depression Scale (CES-D).[38,39] We used the continuous CES-D score (range 0-60) and the dichotomized score (cut-off at 16).

Statistical analyses

Loss to follow-up analyses were conducted by means of chi-square tests and analyses of variance. To examine whether free cortisol levels at baseline were associated with cognitive performance and cognitive decline over a period of six years Linear Mixed Models (LMM) were conducted. With LMM we are able to perform regression analyses with repeated-measures data.[40] This method takes into account the dependency of the repeated observations obtained from the same individual over time. FCI (per standard deviation increase) measured at baseline was assumed to be a potential predictor (main effect) for cognitive performance and cognitive decline over a six year period and was used as independent variable. The repeated measurements of different cognitive domains (general cognitive performance, memory performance and information processing speed) measured at baseline and at 3 and 6 years follow-up were used as the main outcomes. As the MMSE score was not normally distributed, the transformed score ln(31-MMSE) was used to approach normality.

First, we investigated whether the associations between FCI and cognitive performance was different for men and women, young old and old old, and depressed (CES-D $\geq$ 16) and non-depressed (CES-D< 16) persons by entering the product terms of cortisol and these respective variables into the models. An interaction was considered statistically significant when the p-value for the interaction term was below 0.10. When a significant interaction was found, the subsequent analyses were stratified for different groups. Second, to be able to determine whether cortisol levels were associated with the cognitive decline over time, the interaction with time was evaluated by adding the product term between FCI and time to the model (FCI x Time). Third, to investigate the association between FCI and cognitive performance we included in subsequent models several groups of confounders into the analyses in order to show the specific effects of these confounders. Model 1 was adjusted for demographics (age, sex, level of education), model 2 also for health characteristics and lifestyle variables (smoking, alcohol intake, BMI, hypertension, diabetes, cardiovascular disease, and stroke), and model 3 for all previous covariates and depressive symptoms. The unstandardized regression coefficients (B) and their 95% confidence intervals (CI) will be presented.

Finally, the presence of a non-linear association (U-curve) was tested by entering a cortisol-squared term to the fully adjusted models. A non-linear association was considered to be present when the p-value for the cortisol-squared term was below 0.05. From all analyses the 95% confidence intervals are given. SPSS version 14.0 (Chicago, USA) was used to analyze our data.

3. Results

At baseline, the average age of the 1,154 persons with cognition and cortisol measurements was 75.1 years (SD 6.5; range 65-88 years). Table 1 provides the characteristics of the study sample.

First, with LMM we investigated whether the associations between FCI and cognitive performance was different for men and women, young old and old old, and depressed (CES-D $\geq$ 16) and non-depressed (CES-D< 16) persons by entering the product terms into the adjusted models. The results showed no significant interactions with respect to the association between FCI and memory function (learning and retention). However, we found a significant interaction for sex in the association between FCI and MMSE (interaction term: t=2.87, df=1127.19, p=0.004) and information processing speed (interaction term: t=-4.22, df=1124.88, p$\leq$0.001). As a consequence, further analyses on FCI and the MMSE and information processing speed was stratified for sex. No differential effects were found across age and depression status.

Table 1. Characteristics of the study sample (N=1,154)

N	1,154
Female, %	51.4
Age, mean (SD)	75.1 (6.5)
Level of education, mean (SD)	3.6 (2.0)
Hypertension, %	22.3
Cardiovascular disease, %	25.1
Diabetes, %	7.5
Body Mass Index, mean (SD)	26.7 (4.0)
Alcohol use	
No alcohol use, %	22.5
< 14 alcoholic drinks a week, %	55.7
$\geq$ 14 alcoholic drinks a week, %	21.8
Current smoking, %	18.5
Depressive symptoms,	
mean (SD)	7.8 (7.5)
$\geq$ 16, %	13.5
MMSE, mean (SD)	27.2 (2.3)
Memory – learning(total), mean (SD)	19.6 (6.1)
Memory – retention, mean (SD)	68.7 (25.2)
Speed of information processing, mean (SD)	25.8 (7.2)
Free cortisol index (cortisol/CBG), mean (SD)	12.4 (4.6)
Total cortisol, mean (SD)	498.1 (170.8)

Table 2 gives an overview of the associations between FCI per SD increase, for verbal learning and memory retention. High levels of free cortisol were associated with poorer performance on verbal learning (B= -1.29; 95 % CI: -1.62 to -0.96) and memory retention (B= -3.74; 95 % CI: -5.01 to -2.47). This means that per SD increase (4.6) of free cortisol index 1.29 fewer words (out of 15) were learned and 3.74% less of the words that were learned before could be remembered after a distraction period of 20 minutes. After adjustment for demographics, health and lifestyle variables only the association between high levels of free cortisol and poorer performance on verbal learning remained significant, although the strength of this association decreased substantially (B= -0.32; 95%CI: -0.64 to -0.01). Additional adjustment for depression did not change the association between FCI and verbal learning.

Table 2. Associations between free cortisol index and memory functioning (learning and retention)

	Learning (total)					Retention				
	B[a]	95% CI	t	df	p	B[a]	95% CI	t	df	p
Free cortisol index, per SD increase (4.6)	-1.29	-1.62; -0.96	-7.65	1125.41	<0.001	-3.74	-5.01; -2.47	-5.77	1066.74	<0.001
+ adj. for age	-0.85	-1.15; -0.55	-5.53	1133.83	<0.001	-2.43	-3.64; -1.22	-3.93	1076.68	<0.001
+ adj. for demographics[b]	-0.40	-0.70; -0.09	-2.57	1151.39	0.01	-1.29	-2.57; -0.01	-1.97	1086.89	0.05
+ adj. for health and life style[c]	-0.32	-0.64; -0.01	-1.99	1065.06	0.05	-0.92	-2.25; 0.41	-1.36	1004.94	0.17
+ adj. for depression	-0.33	-0.65; -0.01	-2.05	1054.24	0.04	-1.03	-2.36; 0.30	-1.52	996.65	0.13
Free cortisol index*time[d]	-0.02	-0.21; 0.17	-0.21	1660.06	0.83	0.38	-0.63; 1.39	0.74	1686.50	0.46

[a] B = unstandardized regression coefficient
[b] adjusted for age, sex and level of education
[c] adjusted for age, sex, level of education, cardiovascular diseases, hypertension, diabetes, BMI, smoking habits and alcohol consumption.
[d] adjusted for age, sex, level of education, cardiovascular diseases, hypertension, diabetes, BMI, smoking habits, alcohol consumption and depression.

To examine whether FCI was associated with decline in memory function over a period of six years, the interaction with time was evaluated by adding the product term between FCI and time to the fully adjusted models. None of these associations showed significant results, meaning that free cortisol levels are not associated with decline in memory function during a period of six years.

Table 3 shows the associations between FCI and the MMSE in information processing speed for men and women separately, since we found significant sex interactions for these cognition outcomes. The association between FCI and the MMSE is only present in women, and not in men, after adjustment for demographics (B=0.05; 95%CI: 0.01 to 0.10). As the MMSE scores were transformed according to ln(31-MMSE), this means that higher levels of free cortisol are associated with poorer performance on the MMSE. However, after additional adjustment for health and lifestyle variables these associations were no longer significant. In women, we also found a significant association between FCI and information processing speed. Although adjustment for demographics, health and lifestyle variables attenuated this association, it was still significant (B=-0.89; 95%CI: -1.44 to -0.34). Additional adjustment for depression hardly changed this association. Overall, this finding suggests that in women per SD increase of free cortisol (4.6) almost one item per minute less was scored on the speed of information processing task, which might be considered a small decrease.

To examine whether FCI was associated with decline in global cognitive functioning and information processing speed over a period of six years, the interaction with time was evaluated by adding the product term between FCI and time to the fully adjusted models. However, none of these associations showed significant results, meaning that free cortisol levels are not associated with decline in global cognitive functioning and information processing speed over a period of six years.

A non-linear relationship between FCI and cognition was tested by adding a quadratic cortisol term to the final linear mixed models, but none of the quadratic terms reached significance ($p > 0.05$).

Table 3. Associations between free cortisol index and the MMSE[a] and information processing speed for men and women

	MMSE					Information processing speed				
Women	B[b]	95% CI	t	df	p	B[b]	95% CI	t	df	p
Free cortisol index, per SD increase (4.6)	0.13	0.08; 0.18	5.26	588.30	<0.001	-2.23	-2.86; -1.60	-7.01	578.63	<0.001
+ adj for demographics[c]	0.05	0.01; 0.10	2.46	589.97	0.01	-1.10	-1.64; -0.57	-4.06	575.28	<0.001
+ adj. for health and life styled[d]	0.04	-0.01; 0.08	1.58	530.65	0.11	-0.89	-1.44; -0.34	-3.17	517.31	0.002
+ adj. for depression	0.04	-0.01; 0.08	1.59	523.49	0.11	-0.85	-1.40; -0.31	-3.07	512.34	0.002
Free cortisol index*time[e]	-0.01	-0.05; 0.02	-0.78	921.50	0.43	-0.004	-0.24; 0.23	-0.03	789.92	0.97
Men										
Free cortisol index, per SD increase (4.6)	0.03	-0.01; 0.08	1.39	532.24	0.17	-0.38	-0.97; 0.22	-1.25	546.09	0.21
+ adj for demographics[c]	-0.01	-0.05; 0.03	-0.55	541.29	0.58	-0.34	-0.15; 0.83	1.35	552.12	0.18
+ adj. for health and life style[d]	-0.01	-0.05; 0.03	-0.32	530.15	0.75	0.18	-0.31; 0.68	0.73	517.98	0.47
+ adj. for depression	-0.01	-0.05; 0.03	-0.34	498.34	0.73	0.17	-0.32; 0.67	0.69	511.78	0.49
Free cortisol index*time[e]	0.01	-0.03; 0.04	0.38	861.28	0.71	0.13	-0.09; 0.34	1.17	745.69	0.24

[a] MMSE score is transformed ln(31-MMSE) to reach normality – as a consequence lower scores indicate better cognitive performance
[b] B = unstandardized regression coefficient
[c] adjusted for age and level of education
[d] adjusted for age, level of education, cardiovascular diseases, hypertension, diabetes, BMI, smoking habits and alcohol consumption.
[e] adjusted for age, level of education, cardiovascular diseases, hypertension, diabetes, BMI, smoking habits, alcohol consumption and depression.

4. Discussion

In our study we found that high levels of cortisol at baseline were associated with poorer memory function, and in women only, also with slower speed of information processing. Higher baseline levels of cortisol were not associated with a decline of cognitive functioning over a period of six years. The associations between cortisol and cognitive performance were the same for the younger and the older old, and for depressed and non-depressed persons.

In contrast with most other studies, we were able to adjust our analyses for most potential confounders. Our results showed that these adjustments were very important, because the strength of the associations changed considerably when socio-demographics, health and lifestyle variables were entered in the models. Further adjustment for depressive symptoms hardly changed any of the associations, so depression did not appear to be a confounder.

Our finding that high levels of cortisol are cross-sectionally associated with poor memory functions are consistent with earlier studies among younger adults, but also with earlier studies among older persons.[12,13,15-17,19] It seems a very robust finding, especially because there are substantial differences between these studies. For instance, all of these studies used different measures for cortisol: serum cortisol,[12,14,15,18] urinary cortisol[13,19] and salivary cortisol.[16] In addition, studies vary substantially with respect to the number of cortisol measurements and the timing of the measurements. The studies that did not find an association between cortisol and memory function used a visual memory task[18] instead of a verbal memory task as seen in the other studies, or just one question of the MMSE which is not a valid measurement for memory function.[14]

As memory problems are also frequently found in experimental human and animal studies,[2,5,41] it seem reasonable to conclude that high levels of cortisol have a negative impact on memory function. We did not find an association between low levels of cortisol and cognitive function, as suggested by Lupien et al.,[29] but probably a population based sample like ours does not have sufficient power in the lower range of cortisol. In the study of Lupien cortisol levels were suppressed by a pharmacological inhibition of cortisol secretion, and thus reaching lower levels of cortisol than we were probably able to find.

In women, we found that high levels of cortisol were associated with a small decrease in speed of information processing. In older persons, gender differences in the association between cortisol and cognitive impairment are inconsistently found. For instance, Seeman et al.[21] found for women only an association between cortisol and memory performance; whereas others[14,19] found no differences between men and women. It is difficult to explain why these gender differences should exist for speed of information processing and not for memory function. The smaller changes over time in men may have weakened the ability to detect associations between cortisol

and speed of information processing in men. Two other studies found an association between high levels of cortisol and speed of information processing as well,[16,18] but both studies did not investigate whether this association was the same for men and women.

We did not find that the association between cortisol and cognitive performance and cognitive decline was different for persons with and without depressive symptoms. It was important to investigate the role of depression in this respect, as late-life depression is often associated with both, cognitive decline,[23, 24] and high levels of cortisol.[25-28] Apparently, levels of cortisol are associated with memory and information processing speed independently from depression status.

Cortisol and cognitive decline

We did not find an association between baseline cortisol levels and decline in cognitive functioning over the subsequent six years. This is not completely unexpected, because some other studies suggested that cognitive impairment as a consequence of high levels of cortisol are reversible when these levels decrease to normal.[10,11] We had only one baseline measurement of cortisol, and may expect that cortisol levels in a substantial part of our sample will return to normal within the subsequent years. It seems reasonable to assume that only levels of cortisol that stay high during follow-up are related to cognitive decline. For instance, Li et al.[16] showed that persons with poorest memory scores over time were those with increasing levels of cortisol during 3 years of follow-up. Only the study of Karlamangla et al.[19] found that baseline urinary cortisol was predictive for incident cognitive decline in the subsequent 7 years. However, they measured urinary cortisol secretion overnight, which may represent another underlying mechanism than cortisol levels measured during the day. In addition,

HPA-axis hyperactivity is well established in persons with Alzheimer's Disease.[42,43] It would be interesting to further investigate whether overnight levels of cortisol are predictive of dementia, whereas basal cortisol levels during the day have a reversible effect on cognitive function in older persons.

Strengths and limitations

Some methodological aspects of our study need to be addressed. An important strength of our study is that we used a large representative cohort of older persons with data on a wide range variables. This gave us the opportunity to adjust analyses for numerous potential confounders and to look into the role of age, sex and depression. An important shortcoming of our study is, that we have just one cortisol measure. Given the fact that cortisol exhibits a 24-h circadian profile, with a morning maximum 30 minutes after awakening and slowly declining levels during the day and night, it would be preferable to have at least two cortisol measures, one from the morning peak and one from the evening. Additionally, it has also been suggested that cortisol

samples should be collected on several consecutive days,[44] as cortisol levels measured only once may be influenced by acute effects, and not reflect the basal concentration. Nonetheless, we have a very large sample and the cortisol concentrations we found were realistic for the population and the time of the day, and lie well within the normal range.[27] Furthermore, we have no longitudinal data on cortisol three and six years later. Follow-up measurements of cortisol are necessary to further investigate how levels of cortisol covary with cognitive function during follow-up. Then we would also be able to investigate whether cognitive function is reversible when cortisol levels decrease to normal, which is an important issue for further research.

In conclusion, our study provides further evidence that high levels of cortisol measured during the day are predictive for lower memory function and speed of information processing at baseline, but not to a faster decline of cognitive functioning over time.

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3.2 Salivary cortisol, APOE-ε4 allele and cognitive decline in a prospective study of older persons

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Abstract

Objective

We determined whether salivary cortisol levels were associated with cognitive decline at follow-up in older persons and whether this association was modified by the APOE-ε4 allele.

Methods

Within the Longitudinal Aging Study Amsterdam (LASA), a population-based prospective cohort study, 911 persons (74.5±7.2 years, 46.4% male) collected salivary cortisol in the morning and late in the evening. At baseline and after 4 years of follow-up, global cognitive functioning, verbal memory performance, and processing speed were assessed. The longitudinal associations between cortisol measures and cognitive decline were estimated using linear mixed models, adjusted for potential confounders and the modifying role of the APOE-ε4 allele was examined.

Results

Lower morning salivary cortisol levels, higher evening cortisol levels, and flattened diurnal variability of cortisol levels were associated with increased risk for memory decline in APOE-ε4 carriers but not in non-carriers.

Conclusion

Our findings suggest that in this older non-demented population APOE-ε4 carriers may be more vulnerable to the potential detrimental effect of hypothalamic-pituitary-adrenal axis dysfunction on verbal memory performance.

1. Introduction

It has frequently been hypothesized that chronic exposure to glucocorticoids has a damaging effect on the brain and cognitive functioning [1;2]. Biological evidence supporting this hypothesis suggests that increased levels of glucocorticoids are associated with neurodegeneration of the hippocampus [3]. In humans, increased levels of glucocorticoids and reduced glucocorticoid feedback inhibition of the hypothalamic-pituitary adrenal (HPA) axis have been associated with aging [4;5]. Also, neuropsychiatric disorders associated with HPA axis dysfunction, such as depressive disorder, posttraumatic stress disorder and Alzheimer's disease have been found to be associated with smaller hippocampal volumes [6;7]. Based on these findings it has been hypothesized that high levels of cortisol contribute to hippocampal atrophy, cognitive decline and increased risk for Alzheimer's disease [8;9].

Prospective studies on the relation between cortisol levels and cognitive decline have demonstrated highly variable results. Some studies found that high levels of cortisol at baseline [10;11] or increase in cortisol levels over time [12;13] were associated with memory decline or decline in global cognitive functioning. However, other studies did not find that cortisol levels were associated with cognitive decline [14-16]. Contrary to HPA-axis regulation, the apolipoprotein E (APOE)*ε4 allele is a well established risk factor for cognitive decline and Alzheimer's disease [17;18]. APOE is a glycoprotein, which is involved in the transport of cholesterol and other lipids through cell membranes and in the brain it is thought to play a role in cell growth and neuronal regeneration. It has been demonstrated that the APOE-ε4 allele is associated with higher levels of cortisol in CSF in patients with Alzheimer's disease [19]. Also, two recent cross-sectional studies investigated the modifying effect of APOE-ε4 on the association between cortisol levels and cognitive functioning. One of these reported that elevated cortisol levels were associated with poorer cognitive functioning in older persons with two APOE-ε4 alleles but not in heterozygotes or non-carriers[20], while the other study did not find that APOE-ε4 modified this association [21]. These results are highly interesting and it would be of great importance to investigate longitudinally whether the APOE-ε4 allele modifies the association between cortisol levels and cognitive decline.

Therefore, in the present study, we examined firstly whether salivary cortisol levels, collected in the morning and late in the evening, were associated with decline in global cognitive functioning, information processing speed and verbal memory performance in a large population-based cohort of older people with four years of follow-up. Secondly, we examined whether this association was modified by the APOE-ε4 allele. We hypothesized that higher levels of cortisol would be associated with cognitive decline in APOE-ε4 carriers, but not in non-carriers.

2. Methods

LASA

Data were used from the Longitudinal Aging Study Amsterdam (LASA). LASA is a population-based prospective cohort study among 3,107 persons initially aged 55–85 years in the Netherlands that started in 1992/1993 (T1). Details of this cohort study have been described previously [22]. For this study, data were used from the third follow-up (T4) in 2001-2002 when 1,691 subjects participated (81% of T3) and the fourth follow-up (T5) in 2005-2006 when 1,266 subjects participated (74% of T4). Of the 1,416 participants who were lost to follow-up between T1 and T4 1,050 (33.8 % of the T1 sample) had died, 112 (3.6 %) indicated by self-report or proxy that they were too ill or cognitively impaired to be interviewed, 222 (7.1 %) indicated that they were no longer interested in participating in the study, and 32 (1.0 %) could not be contacted.

Cortisol measurement

Saliva samples were collected with the "Salivette" device from Sarstedt, in Nümbrecht, Germany. Materials for saliva sampling and detailed instructions were sent home to the participants. Participants had to collect saliva within 30 minutes after awakening and just before going to bed. In order to prevent contamination with blood, participants were asked not to eat or brush their teeth and had to rinse their mouth and wait ten minutes before starting to chew the cotton ball [23]. The samples were kept in the refrigerator until collected by the interviewer the next day.

In the laboratory, the saliva samples were centrifuged and stored at –20 C until analysis.

Cortisol levels (nmol/L) were determined using radio immunoassay coated tubes (Spectria Orion Diagnostics, Finland). The detection limit was 1.5 nmol/L. The intra-assay coefficient of variation was 7% for cortisol levels around 30 nmol/L and 19% for cortisol levels around 1.3 nmol/L, whereas corresponding inter-assay coefficients of variation were 5% and 19% [24].

Cognition

Global cognitive functioning was determined using the Mini Mental State Examination (MMSE) [25]. Because MMSE scores had a skewed distribution, we computed the MMSE score into a reverse score and applied natural log transformation: ln(31-MMSE score)[26], to obtain a normal distribution.

Verbal memory performance was tested with the 15-word learning test [27;28], in which 15 unrelated words had to be learned over three consecutive trials. After each trial participants had to recall as many words as possible (immediate recall). After a distraction period of 20 minutes, participants were asked to recall the words they had learned during the three trials. The retention score was calculated by dividing the delayed recall score by the maximum number of words recalled during the immediate recall.

Information processing speed was tested using an adjusted version of the digit-coding task [29]. In this task two rows of characters were shown. Each character in the first row belongs to a character in the second row. Participants had to complete as many character combinations as possible by verbally naming the corresponding character. This test was repeated over three trials of one minute each. The trial in which the respondent gave the most answers was included as the maximum score.

APOE genotype determination

Serum samples collected at T2 and T3 were frozen at -80° C until *APOE* genotypes were determined by isoelectric focusing of delipidated plasma samples, followed by immunoblotting. Participants were classified as *APOE*-ε4 carriers for those with an *APOE*-ε4 isoform (genotypes ε2/4 ε3/4 ε4/4) and as non-carriers for those without an *APOE*-ε4 isoform (genotypes ε2/2, ε2/3, ε3/3) [30].

Other variables

Age, gender and level of education were recorded during the baseline interview of LASA (T1). Educational level was recorded in seven categories ranging from 6 years of education or less to college or university education. At T4 alcohol consumption, smoking habits, hours of sleep, history of diabetes, atherosclerosis, heart disease, and stroke were assessed with self-report questionnaires. Alcohol consumption was expressed as number of drinks per week and was categorized in three groups (no alcohol use; on average <14 drinks weekly; 14 drinks weekly). Smoking habits were categorized in current and non-current smoker. Medication use was assessed by self-report and inspection of the medicine containers [31]. Blood pressure was measured twice, and the average of the two measurements was calculated. Hypertension was defined as a diastolic tension >90 mmHg or a systolic tension above 140 mmHg or use of anti-hypertensive medication. Body Mass Index (BMI) was calculated from measured weight and height and expressed as kg/m^2. Depressive symptoms were measured using the Dutch version of the Center for Epidemiologic Studies Depression scale (CES-D) [32].

Study sample

Of the 1,691 persons participating at T4, saliva was collected by 1,184 participants, of whom 1,010 persons had data on APOE genotype. From these, 27 participants (2.7 %) used corticosteroids and were excluded from the study sample. Participants with possible dementia (n=23) were also excluded from the study sample. Possible dementia was defined as a persistent decline in MMSE score of more than 2 standard deviations from the mean over three consecutive measurements and by indication of a proxy [26]. Of the saliva samples, 21 morning samples and 17 evening samples were excluded because of insufficient volume of saliva or improbable cortisol levels (cortisol levels higher than 4 standard deviations above mean morning cortisol) [24]. Missing data on covariates existed for 32 participants, leaving 911 participants for

data-analysis. Of these, complete data was available for 698 participants for follow-up measurements four years later.

Data analysis

First, baseline characteristics were calculated according to tertiles of diurnal variability levels, calculated as the absolute difference between morning and evening levels. Differences in baseline characteristics were analyzed using t-tests for normally distributed data and Chi-square and Mann-Whitney tests for non-normally distributed data.

Second, the longitudinal association between salivary cortisol and cognitive decline was estimated using random coefficient analyses, in which the rate of change in cognitive functioning over time (i.e. intercepts and slopes) was fitted as random effects. This method takes into account multiple observations per subject that are likely to be correlated. The different cognition measures were entered as dependent variables and the cortisol measures were entered as independent variables per standard deviation increase. The main effects of cortisol and age (as the time variable) were entered as continuous variables. Age was centered around the mean age of the study population at baseline (75.5 years), to obtain intercepts that represented the mean score on the respective cognitive test. To estimate the rate of change in cognition over time (age) as a function of cortisol levels interaction terms between cortisol and age were entered in the same model. Analyses were adjusted for sex, level of education, diabetes, atherosclerosis, heart disease, stroke, hypertension, BMI, mean hours of sleep, smoking and alcohol habits, APOE-ε4 (one or two ε4 alleles vs. non-carriers) and depressive symptoms. The models with diurnal variability as the dependent variable were also adjusted for morning levels of cortisol, to obtain relative diurnal measures.

Third, to examine whether APOE-ε4 modified the association between cortisol and cognitive decline we entered interaction terms between cortisol measures, APOE ε4 and time to the fully adjusted models, including the interaction terms nested within this interaction. Finally, we repeated all analyses after excluding APOE-ε2 carriers (N=113).All analyses were carried out in Statistical Analysis System (SAS), version 9.1 (Cary, NC, USA).

3. Results

Table 1 presents the characteristics of the study sample (N=911), for which complete data was available, and the 780 subjects who were excluded because of nonresponse and incomplete data. Compared with those who were excluded, the participants in the study sample were significantly younger, more often male, were less often current smokers, had less often CVA, diabetes and an APOE-ε4 allele and had better memory functioning and higher levels of morning cortisol ($p < 0.05$).

Between baseline and our follow-up measurement 213 participants were lost to follow-up. Loss to follow-up was associated with significantly higher levels of evening cortisol, but not with awakening cortisol, diurnal variability of cortisol or APOE-ε4.

Table 1. Characteristics of the study sample and subjects excluded from the analysis because of nonresponse or missing data

	Study sample	Subjects excluded
N	911	780
Mean age (sd)	75.5 (6.8)	77.6 (8.9)*
Male (%)	46.7	37.4*
Mean level of education (sd)[a]	3.7 (2.0)	3.4 (2.0)
Mean body mass index (sd)	27.4 (4.2)	27.5 (4.3)
Atherosclerosis (%)	10.1	12.2
Heart disease (%)	29.8	29.2
Cerebrovascular disease (%)	7.0	11.7*
Hypertension (%)	31.6	29.7
Diabetes (%)	9.6	12.4*
Current smoker (%)	14.3	17.1*
Alcohol intake		
No alcohol use (%)	19.3	23.1
< 14 drinks per week (%)	59.5	55.4
≥ 14 drinks per week (%)	21.5	21.2
Median depression score (10-90 %)[b]	7.0 (1.0 – 18.0)	7.0 (1.0 – 20.0)
Median morning cortisol (10-90 %)	15.0 (7.4 – 27.0)	14.0[¶,*] (7.0 – 24.0)
Median evening cortisol (10-90 %)	2.9 (1.5 – 5.7)	2.9[¶] (1.6 – 6.6)
Median MMSE score (10-90 %)	28.0 (25.0 – 30.0)	28.0 (23.0 – 30.0)
Mean digit-coding test score (sd)	26.5 (7.2)	25.3 (7.8)
Mean AVLT immediate recall (sd)	21.0 (6.3)	18.7 (7.2)*
Mean AVLT delayed recall (sd)	6.4 (3.0)	5.5 (3.3)*
Mean AVLT retention (sd)	70.6 (24.0)	63.4 (28.5)*
APOE-ε4 carriers (%)	25.1	30.8[#]

*: $p < 0.05$ tested with t-tests for normally distributed data and Chi-square and Mann-Whitney tests for non-normally distributed data.
¶: number of participants of whom cortisol data was available excluded subjects is 306
#: number of participants of whom data on APOE-e4 genotyping was available in excluded subjects is 451

Table 2. Baseline characteristics of the study sample per tertile of diurnal variability of cortisol

	Tertiles of diurnal variability of cortisol			
	≤ 8.6 nmol/L	**8.61 – 14.0 nmol/L**	**≥ 14.01 nmol/L**	**p-value***
N	323	283	305	
Mean age (sd)	76.0 (7.2)	75.7 (6.6)	74.7 (6.6)	0.09
Male (%)	50.3	49.5	41.0	0.02
Mean level of education (sd)[a]	3.7 (2.0)	3.8 (2.0)	3.7 (2.0)	0.85
Mean body mass index (sd)	27.9 (4.1)	27.2 (4.2)	27.2 (4.4)	0.10
Atherosclerosis (%)	9.3	10.2	9.9	0.86
Heart disease (%)	30.5	29.3	27.9	0.81
Cerebrovascular disease (%)	9.6	6.4	4.8	0.06
Hypertension (%)	29.4	31.8	32.4	0.44
Diabetes (%)	13.6	7.1	8.0	0.02
Current smoker (%)	15.6	13.8	12.2	0.57
Alcohol intake				
No alcohol use (%)	20.9	18.0	19.2	0.64
< 14 drinks per week (%)	56.6	62.9	58.7	0.25
≥ 14 drinks per week (%)	22.5	19.1	22.1	0.51
Median depression score (10-90 %)[b]	8.0 (1.0 – 19.0)	7.0 (1.0 – 17.6)	7.0 (1.0 – 17.7)	0.37
Median morning cortisol (10-90 %)	9.0 (5.6 – 13.0)	14.0 (11.0 – 17.0)	22.0 (17.0 – 33.0)	0.00
Median evening cortisol (10-90 %)	3.1 (1.6 – 6.9)	2.7 (1.5 – 5.0)	2.8 (1.5 – 5.2)	0.00
Median MMSE score (10-90 %)	27.5 (24.0 – 30.0)	28.0 (25.0 – 30.0)	28.0 (25.0 – 30.0)	0.08
Mean digit-coding test score (sd)	26.1 (7.2)	26.9 (7.0)	27.4 (7.0)	0.04
Mean AVLT immediate recall (sd)	20.0 (6.3)	21.0 (6.3)	22.1 (6.1)	0.00
Mean AVLT delayed recall (sd)	6.1 (3.0)	6.4 (3.0)	6.9 (3.0)	0.00
Mean AVLT retention (sd)	70.2 (24.1)	70.4 (23.8)	73.2 (22.3)	0.22
APOE-ε4 carriers (%)	25.4	25.4	24.6	0.98

*: tested with t-tests for normally distributed data and Chi-square and Mann-Whitney tests for non-normally distributed data.

[a] Educational level ranges from 0 (less <= 6 years of education) to 9 (university).

[b] Depression score measured with Center for Epidemiological Studies Depression Scale (0-30).

In our study sample the overall mean age was 75.5 ± 6.8 years, 47% was male, median morning and evening cortisol levels were 15.0 (10-90 %: 7.5 – 17.0) nmol/L and 2.9 (10-90 %: 1.5 – 5.6) nmol/L respectively, and 25% were APOE-ε4 carriers. Table 2 shows the baseline characteristics of the study sample according to tertiles of diurnal variability of cortisol. Participants in the lowest tertile of diurnal variability were somewhat older, more often male, had more often diabetes and cerebrovascular diseases and had lower scores on cognitive tests than participants in higher tertiles ($p< 0.05$).

Table 3 presents the adjusted associations between cortisol measures and cognition measures. Scores on all cognitive tests declined over time, but salivary cortisol levels were not associated with decline over time on any of these tests, although significant main effects were observed for higher evening cortisol levels and flattened diurnal variability with poorer memory performance, suggesting that increased evening levels at baseline was associated with poorer memory performance at baseline as well as at follow-up but not with more decline in memory performance.

Table 3. Adjusted associations between cortisol measures and cognition scores for whole study sample.

	Global cognitive functioning [a]	**Information processing speed [b]**
	B (95% CI)	**B (95% CI)**
Morning cortisol (per 1 SD increase [e])	0.02 (-0.02 to 0.05)	0.14 (-0.27 to 0.55)
Intercept	28.97 (28.70 to 29.25)	26.61 (23.52 to 26.69)
Time	-0.03 (-0.04 to -0.02)*	-0.36 (-0.46 to -0.26)*
Time*morning cortisol [d]	0.00 (-0.01 to 0.00)	-0.01 (-0.06 to 0.03)
Evening cortisol (per 1 SD increase [f])	0.03 (0.00 to 0.06)	-0.18 (-0.57 to 0.21)
Intercept	29.01 (28.75 to 29.26)	27.24 (24.32 to 30.16)
Time	-0.03 (-0.03 to -0.02)*	-0.38 (-0.44 to -0.32)*
Time*evening cortisol [d]	0.00 (0.00 to 0.01)	0.01 (-0.05 to 0.04)
Diurnal variability (per 1 SD increase [g])	0.01 (-0.07 to 0.10)	0.22 (-0.77 to 1.22)
Intercept	28.97 (28.70 to 29.34)	26.80 (23.71 to 26.89)
Time	-0.03 (-0.04 (-0.02)*	-0.39 (-0.48 to 0.30)*
Time*diurnal variability [d]	0.00 (-0.01 to 0.00)	0.00 (-0.04 to 0.05)

Table 3. Adjusted associations between cortisol measures and cognition scores for whole study sample - *continued*

	Verbal memory [c] Immediate recall	Verbal memory [c] Delayed recall	Verbal memory [c] Memory retention
	B (95% CI)	**B (95% CI)**	**B (95% CI)**
Morning cortisol (per 1 SD increase [e])	0.18 (-0.02 to 0.05)	0.02 (-0.16 to 0.19)	-0.72 (-2.07 to 0.63)
Intercept	23.06 (20.30 to 25.83)	7.57 (6.20 to 8.93)	79.89 (69.19 to 90.59)
Time	-0.37 (-0.47 to -0.26)*	-0.17 (-0.22 to -0.11)*	-0.93 (-1.40 to -0.47)*
Time*morning cortisol [d]	-0.01 (-0.06 to 0.04)	0.00 (-0.04 to 0.02)	0.05 (-0.16 to 0.25)
Evening cortisol (per 1 SD increase [f])	-0.20 (-0.55 to 0.13)	-0.20 (-0.37 to -0.03)*	-1.55 (-2.81 to -0.28)*
Intercept	23.83 (21.22 to 26.43)	7.81 (6.52 to 9.08)	79.23 (69.09 to 89.36)
Time	-0.37 (-0.43 to -0.31)*	-0.16 (-0.20 to -0.13)*	-0.70 (-0.97 to -0.42)*
Time*evening cortisol [d]	0.00 (-0.04 to 0.05)	0.00 (-0.02 to 0.02)	-0.16 (-0.36 to 0.05)
Diurnal variability (per 1 SD increase [g])	0.73 (-0.17 to 1.64)	0.54 (0.11 to 0.99)*	4.15 (0.44 to 7.87)*
Intercept	23.20 (20.44 to 25.96)	7.69 (6.33 to 9.05)	80.70 (70.02 to 91.39)
Time	-0.39 (-0.48 to -0.27)*	-0.18 (-0.22 to -0.14)*	-1.06 (-1.46 to -0.66)*
Time*diurnal variability [d]	0.01 (-0.03 to 0.06)	0.01 (-0.01 to 0.03)	0.15 (-0.05 to 0.36)

Associations are adjusted for sex, levels of eductaion, atherosclerosis, heart disease, cerebrovascular diseases, hypertension, diabetes, BMI, smoking and drinking habits, APOE-ε4 and depressive symptoms.

* $p < 0.05$

[a] As measured with Mini Mental State Examination

[b] As measured with Digit-coding test

[c] As measured with 15-word learning test

[d] time is represented by mean age at baseline; the interaction term reflects the rate of cognitive decline over time

[e] SD morning cortisol: 8.07 nmol/L

[f] SD evening cortisol: 4.36 nmol/L

[g] SD diurnal variability: 7.47 nmol/L

When we tested interactions between cortisol measures, APOE-ε4 and time, significant interactions were found for morning cortisol*APOE-ε4*time on immediate recall (B= 0.18; 95% CI 0.05 to 0.304, p< 0.01); diurnal variability *APOE-ε4*time on immediate recall (B= 0.19; 95% CI 0.07 to 0.31, p< 0.01); evening cortisol *APOE-ε4 time on delayed recall (B= 0.09; 95% CI 0.01 to 0.17, p= 0.02); and diurnal variability *APOE-ε4*time on delayed recall (B= 0.05; 95% CI 0.00 to 0.11, p= 0.08). Table 4 presents the adjusted associations of cortisol measures with immediate and delayed recall stratified for APOE-ε4. In the APOE-ε4 carriers, but not in non-carriers, lower morning cortisol and flattened diurnal variability were associated with more decline on immediate recall score. Also, in APOE-ε4 carriers but not in non-carriers, higher evening cortisol and flattened diurnal variability were associated with more decline on delayed recall score. Figures 1 and 2 present these results of the random coefficient analyses with cortisol measures categorized into tertiles, for immediate recall and delayed recall respectively. When we excluded the APOE-ε2 carriers from the sample the results did not materially change (data not shown).

Table 4. Adjusted associations between cortisol measures and verbal memory scores, stratified for *APOE*-ε4 carriers and non-carriers

	Verbal memory – immediate recall [a]		Verbal memory – delayed recall [a]	
	APOE-ε4 carriers	APOE-ε4 non-carriers	APOE-ε4 carriers	APOE-ε4 non-carriers
	B (95% CI)	B (95% CI)	B (95% CI)	B (95% CI)
Morning cortisol				
(per 1 SD increase [c])	-0.13 (-0.90 to 0.63)	0.29 (0.12 to 0.71)*	-0.17 (-0.54 to 0.21)	0.08 (-0.13 to 0.28)
Intercept	26.67 (20.70 to 32.64)	21.61 (18.46 to 24.72)	9.96 (7.03 to 12.90)	6.68 (5.14 to 8.22)
Time	-0.69 (-0.94 to -0.44)*	-0.29 (-0.41 to -0.17)*	-0.28 (-0.41 to -0.16)*	-0.13 (-0.19 to -0.08)*
Time*morning cortisol [b]	0.14 (0.02 to 0.25)*	-0.04 (-0.09 to 0.01)	0.04 (-0.01 to 0.10)	-0.01 (-0.04 to 0.01)
Evening cortisol				
(per 1 SD increase[d])	0.03 (-0.98 to 1.05)	-0.22 (-0.60 to 0.15)	0.21 (-0.29 to 0.71)	-0.21 (-0.40 to -0.02)*
Intercept	26.43 (20.88 to 31.98)	22.69 (19.73 to 25.64)	9.29 (6.54 to 12.04)	7.12 (5.67 to 8.57)
Time	-0.43 (-0.60 to -0.26)*	-0.36 (-0.43 to -0.28)*	-0.26 (-0.35 to -0.18)*	-0.15 (-0.18 to -0.12)*
Time*evening cortisol [b]	0.04 (-0.13 to 0.21)	0.00 (-0.04 to 0.05)	0.09 (0.01 to 0.17)*	-0.01 (-0.03 to 0.01)
Diurnal variability				
(per 1 SD increase [e])	1.40 (-1.95 to 4.75)	0.68 (-0.28 to 1.64)	0.40 (-1.26 to 2.07)	0.57 (0.10 to 1.03)*
Intercept	26.32 (20.31 to 32.32)	21.92 (18.81 to 25.03)	9.95 (6.96 to 12.93)	6.88 (5.35 to 8.40)
Time	-0.68 (-0.90 to -0.47)*	-0.32 (-0.43 to -0.22)*	-0.28 (-0.39 to -0.17)*	-0.15 (-0.20 to -0.11)*
Time*diurnal variability [b]	0.17 (0.06 to 0.30)*	-0.02 (-0.07 to 0.03)	0.05 (-0.01 to 0.11)*	0.00 (-0.03 to 0.02)

Associations are adjusted for sex, levels of educations, atherosclerosis, heart disease, cerebrovascular diseases, hypertension, diabetes, BMI, smoking and drinking habits and depressive symptoms.

* $p < 0.05$

[a] As measured with 15-word learning test

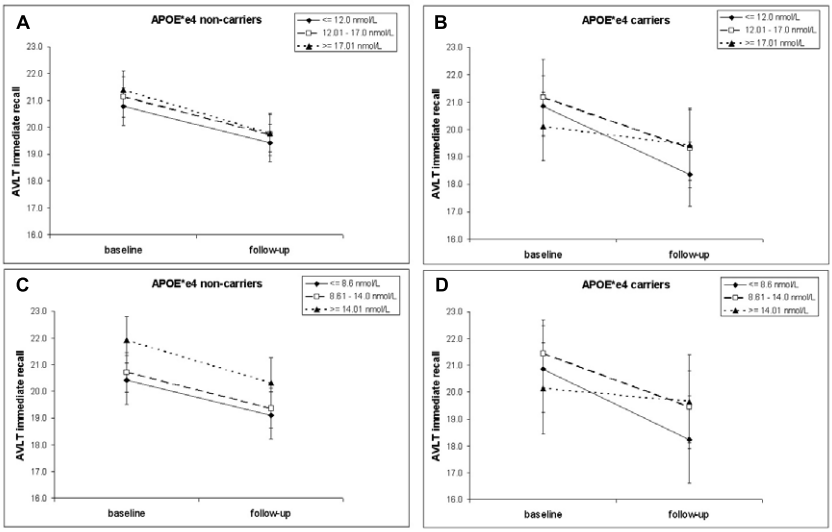
[b] time is represented by mean age at baseline; the interaction term reflects the rate of cognitive decline over time

[c] SD morning cortisol: 8.07 nmol/L

[d] SD evening cortisol: 4.36 nmol/L

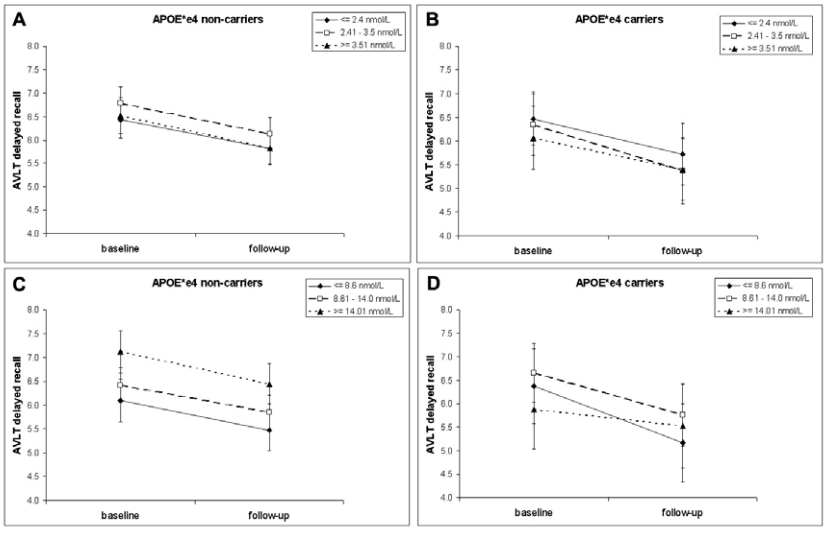
[e] SD diurnal variability: 7.47 nmol/L

Figure 1. Adjusted immediate recall scores per tertiles of morning cortisol and diurnal variability of cortisol, stratified for APOE*ε4 carriers and non-carriers.



A and B: morning cortisol
C and D: diurnal variability of cortisol

Figure 2. Adjusted delayed recall scores per tertiles of evening cortisol and diurnal variability of cortisol, stratified for APOE*ε4 carriers and non-carriers.



A and B: evening cortisol
C and D: diurnal variability of cortisol

4. Discussion

In this large community-based population of older persons, we observed that lower morning cortisol levels, higher evening cortisol levels, and flattened diurnal variability of cortisol levels were associated with increased risk for memory decline in APOE-ε4 carriers but not in non-carriers. Cortisol levels were not associated with decline in global cognitive functioning or information processing speed, neither in APOE-ε4 carriers nor in non-carriers.

Strengths of this study are that we investigated the relation between cortisol and cognitive decline in a prospective design and in a large and population-based cohort of older persons. Furthermore, we had data on several cognitive domains, which made it possible to study not only memory functioning but global cognitive functioning and information processing speed as well. Additionally, we were able to adjust the relations for a large number of potential confounders.

A limitation of our study is that we do not know exactly at which time the saliva samples were taken, as the participants were asked to collect saliva within 30 minutes after awakening instead of directly at awakening or 15 or 30 minutes after awakening. Cortisol follows a specific circadian rhythm, with peak levels 30 minutes after awakening and a nadir at night. Consequently, it is unclear whether the morning samples in our study reflect the awakening level, the peak at 30 minutes after awakening, or any time within this 30 minute interval. Although this will have lead to misclassification, we find it unlikely that it was systematic, i.e. that participants who showed cognitive decline during follow-up selectively collected saliva either at awakening or 30 minutes after awakening. Rather, we think this has lead to random misclassification, which will have lead to dilution of estimates. Additionally, it is recommended to collect cortisol samples on two consecutive days [33], because cortisol levels are rather variable within individuals. However, our large sample size may have largely compensated for the possible variability in cortisol measures.

Two previous recent studies also examined the relation between cortisol levels and cognitive performance in APOE-ε4 carriers and non-carriers. One study found that in APOE-ε4 carriers, but not in non-carriers, cortisol levels were associated with poorer cognitive functioning [20], but in the second study no differences were found between *APOE*-ε4 carriers and non-carriers[21]. Although our findings are in line with the first study, both studies differed from ours on several methodological aspects. For instance, in the latter study 24-hour serum cortisol was collected in a small sample (n=17) [21], while in the first study cortisol levels were measured during cognitive testing, as a measure of stress [20].

To our knowledge, this is the first study to examine the modifying role of in *APOE*-ε4 the relation between cortisol levels and cognitive decline using a prospective design. The findings of increased risk for memory decline but not decline in global cognitive functioning or information processing speed are consistent with a previous

study, which reported that APOE-ε4 carriers predominantly show atrophy of brain structures which are related to memory performance [34]. Results from animal studies show that glucocorticoid receptors are distributed in brain structures, such as the limbic system, hypothalamus and prefrontal cortex [35;36]. Therefore, memory performance is more likely to be affected by cortisol than cognitive functions that do not rely on specific brain structures, such as information processing speed and global cognitive functioning [37]. It is also possible, however, that we did not find an association with decline in global cognitive functioning because our study sample showed a relatively small decline on the MMSE making it more difficult to find a significant association. Also, the MMSE is a relatively insensitive measure of global cognitive impairment in non-demented elderly and it may have been more difficult to detect subtle changes in cognitive functioning.

In our study frontal lobe functions, like executive functioning, were not measured, whereas it has been suggested that particularly frontal lobe functions are sensitive to the effects of glucocorticoids [1] and aging [38]. So it remains unresolved to which extent cortisol levels are associated with executive functioning in APOE-ε4 carriers.

We observed that higher levels of evening cortisol were associated with more rapid decline on delayed recall, and that lower levels of morning cortisol were associated with more rapid decline on immediate recall. Delayed recall is usually considered to be a hippocampal dependent memory function [39], whereas immediate recall also relies on cognitive functions such as attention [40], which are not located in specific brain areas. Mineralocorticoid receptors (type I) are mainly distributed in the hippocampus, whereas the glucocorticoid (type II) receptors are also distributed in other areas of the brain, such as the prefrontal cortex[35]. These two receptor types not only differ in location of distribution, but also in binding affinity. Type I receptors bind glucocorticoids with an affinity which is 6- to 10 times higher than that of type II receptors. As a consequence, type I receptors are occupied when glucocorticoid levels are low to moderate, for instance in the evening, whereas type II receptors are only occupied when glucocorticoid levels are higher, as is usually the case in the morning and during stressful circumstances[41]. Since the hippocampus plays an inhibitive role in HPA axis regulation [42] and type I receptors are mainly distributed in the limbic system, it is possible that the association between high evening cortisol levels and decline on delayed recall is a result of early hippocampal atrophy [43].

Our finding of lower morning cortisol and more decline on immediate recall is more difficult to explain since the majority of studies reported poorer memory functioning in relation to higher cortisol levels. Although, so far no previous study reported [11] or found [16] an association between memory decline and morning cortisol, but only with evening cortisol [11], 24-hour cortisol [13] and with cortisol responses to experimental stress [44]. Lower morning cortisol levels have however been found in patients with hippocampal atrophy [45] and in patients with severe amnesia and possible medial temporal lobe atrophy [46]. Furthermore, it is also possible that not only elevated cortisol levels, but also lower levels may increase risk for memory decline. This is in line with the hypothesis that memory performance is optimal during moderate levels

of glucocorticoids [47] when the ratio of type I/type II receptor occupation is highest [48].

Although our findings of higher evening cortisol with more decline on delayed recall and lower morning cortisol with more decline on immediate recall may seem discrepant; it should be noted that the direction of the associations was the same for lower morning levels of cortisol with delayed recall and for higher evening levels and immediate recall. Moreover, flattened diurnal variability was significantly associated with more rapid decline on both immediate and delayed recall. It is thus possible that the diurnal variability of cortisol is of importance, rather than morning or evening levels by itself. It has been suggested that the diurnal variability is a good marker for HPA axis functioning [49;50] and that flattening of the diurnal variability is associated with aging, frailty, memory complaints and several somatic diseases [50-55].

Our finding that cortisol levels increased risk for memory decline only in APOE-ε4 carriers could also suggest that APOE-ε4 carriers are more vulnerable to potential detrimental effects of dysfunction of the HPA-axis on the hippocampus. Also, the modifying role of APOE-ε4 may suggest that glucocorticoids do not play a direct causal role in hippocampal atrophy and memory decline, but that a subgroup of APOE-ε4 carriers have an early stage of Alzheimer's disease although clinical symptoms are not yet present. If true, this would suggest that our findings may be explained by a shared underlying early Alzheimer's disease process [56].

In conclusion, in this older non-demented population lower morning cortisol levels, higher evening levels and flattened diurnal variability of cortisol were associated with increased risk of memory decline in APOE-ε4 carriers but not in non-carriers. Future studies should determine whether HPA-axis dysfunction in APOE-ε4 carriers plays a role in the development of Alzheimer's disease.

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3.3 Childhood and recent life events, HPA-axis activity, and cognitive functioning. The SMART-Medea study

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Abstract

Objective

We investigated whether the experience of childhood and recent events was associated with cognitive functioning and whether HPA-axis activity explained this relation.

Method

In 736 patients (mean age 62±9 years; 81% male) with a history of atherosclerotic disease from the SMART-Medea study, an ancillary study to the SMART-MR study, memory performance, executive functioning and processing speed/attention were assessed and expressed in Z-scores. Childhood abuse, childhood events and recent life events were assessed using questionnaires and HPA-axis activity was assessed by four morning saliva samples, two evening samples and one awakening sample after 0.5 mg dexamethasone. Cross-sectional analyses were performed using generalized linear models, adjusted for demographics and cardiovascular risk factors.

Results

Participants with childhood abuse had significantly better memory performance (mean difference=0.16; 95% CI 0.00-0.32; p=0.05) and better processing speed/attention (mean difference=0.18; 95% CI 0.03-0.34; p=0.02). Childhood events were not associated with any of the cognitive domains. Participants with more recent events had better executive functioning (mean difference= 0.18; 95% CI 0.02 to 0.35; p=0.04). HPA-axis activity was not associated with cognitive functioning.

Conclusions

Childhood abuse and to a lesser extent recent life events were associated with better cognitive functioning. HPA-axis activity was not associated with cognitive functioning, and could therefore not explain the relation between stressors and cognitive functioning.

1. Introduction

It has been suggested that the experience of stressful life events leads to cognitive impairment.[1-3] For instance, the experience of childhood events has been associated with poorer cognitive functioning later in adulthood.[4-6] The experience of life events later in life has also been associated with cognitive impairment,[7] and cognitive decline[3] although enhanced cognitive functioning has also been found. [8]

It has been hypothesized that dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis is the underlying mechanism in this relation. Stressful life events would lead to long-term changes in HPA-axis regulation[9;10] and dysregulation of the HPA-axis would lead to cognitive impairment and cognitive decline.[1;11] Findings from experimental studies suggest that particularly stress in the morning and high activation of the HPA-axis to stressors will result in poorer memory performance. [12;13] However, the possible moderating role of HPA-axis activity in the relation between stress and cognitive functioning has hardly been examined in observational studies[3;14] and findings are not consistent. One study found no support for a mediating role of cortisol,[3] whereas another study reported that low cortisol levels during exam stress were associated with better memory performance but poorer attention.[14]
The aim of this study was threefold. First, we examined the associations of childhood abuse, childhood events and life events later in life with cognitive functioning. Secondly, we examined the separate associations of life events with HPA-axis activity and HPA-axis activity with cognitive functioning, and thirdly, we investigated to what extent HPA-axis activity explained or mediated the relation between life events and cognitive functioning. We did so in a large cohort of participants with a history of atherosclerotic disease, because stressful life events increase the risk for cardiovascular diseases[15] and these participants have increased risk for HPA-axis dysregulation,[16] and cognitive impairment.[17] They may thus be more vulnerable to the effects of life events and HPA-axis activity on cognitive functioning.

2. Methods

Subjects

Data were used from the Second Manifestations of ARTerial disease-Magnetic Resonance (SMART-MR) study, a prospective cohort study aimed to investigate brain changes on MRI in 1309 independently living patients with symptomatic atherosclerotic disease. Details of the design and participants have been described elsewhere.[18] In brief, between May 2001 and December 2005, all patients newly referred to the University Medical Center Utrecht with manifest coronary artery disease, cerebrovascular disease, peripheral arterial disease or an abdominal aortic aneurysm (AAA), and without MR contraindications were invited to participate. During a 1-day visit to our medical center, an MRI of the brain was performed, in addition to a physical

examination, ultrasonography of the carotid arteries, and blood and urine sampling. Risk factors, medical history, and functioning were assessed with questionnaires that the patients completed before their visit to the medical centre.

Between January 2006 and May 2009, all participants still alive were invited for follow-up measurements, including MRI of the brain, neuropsychological testing, a physical examination, blood and urine sampling, risk factors, medical history, and functioning. In addition, as part of the SMART-Medea (Memory, depression and aging) study, an ancillary study to the SMART-MR study, aimed to investigate brain changes associated with psychosocial vulnerability and stress factors, measurements of salivary cortisol and psychosocial stressors early and later in life were added. The SMART-MR and SMART-Medea study were approved by the ethics committee of our institution and written informed consent was obtained from all participants.

In total, 754 patients of the surviving cohort (61% of n=1,238) gave written informed consent and participated at follow-up; 466 (38%) persons refused and 18 (1%) were lost to follow-up. Of the 754 patients who were examined between 2006 and 2009, the assessment of cognitive functioning was done in 736 patients.

Life events

Childhood abuse was assessed using a structured inventory of childhood abuse and neglect before the age of 16 years. Childhood events were assessed by asking whether patients had experienced stressful events before the age of 16 years[19] and whether they had been separated from their mother of at least 3 months before the age of 12 years.[20] Recent life events in the past 12 months were assessed using a structured questionnaire[21] (table 2).

Neuropsychological assessment

Cognitive functioning was assessed with a set of standard neuropsychological tests, sensitive to mild impairments. We assessed memory, executive functioning and processing speed and attention. For each of these cognitive domains composite z-scores were computed by averaging the z-scores of all subtests per domain.

Verbal memory was assessed with 5 consecutive trials of the 15-word learning test (a modification of the Rey Auditory Verbal Learning test).[22] Immediate recall and delayed recall were assessed. Non-verbal memory was assessed using the delayed recall of the Rey-Osterrieth Complex Figure test.[23]

Executive functioning was assessed with three tests. The visual elevator test (subtest of the Test of Everyday Attention[24]) is a timed test of 10 trials that measures mental flexibility and shifting of attention. The Brixton Spatial Anticipation test[25] was used to assess the capacity to discover logical rules and mental inhibition and flexibility. The total number of errors made was scored. The Verbal Fluency test (letter A, one minute time frame) was used to assess mental flexibility and employment of strategies. Before calculating the z-scores, the scores of the Visual Elevator test and

Brixton Spatial Anticipation test were multiplied by minus one, so that lower scores represented poorer performance.

Processing speed and attention were measured using the Digit Span Forward and Digit Span Backward. In total there were 16 trials and the maximum of correct trials was scored. Also, the Symbol substitution test (subtest from the Wechsler Adult Intelligence Scale) was administered and the number of correct responses during a two minute time frame was scored. Finally, global cognitive functioning was measured using the mini-mental state examination (MMSE).[26]

HPA-axis activity

HPA-axis activity was assessed by 7 measurements of cortisol in saliva over a period of 24 hours to obtain the circadian rhythm. The saliva was collected using cotton dental rolls (Salivette, Startstedt). Participants were instructed to refrain from smoking, drinking caffeine, eating or cleaning their teeth at least 30 minutes before collecting a saliva sample, and to chew on the rolls for at least 2 minutes. On day 1, participants were instructed to take the first sample immediately after awakening while still lying in bed, and to take the second, third and fourth samples after 30, 45 and 60 minutes. Sample 5 and 6 were collected at 10PM and 11PM, respectively. Furthermore, participants were asked to take 0.5 mg of dexamethasone orally after their sixth saliva sample, and to sample their saliva the next morning directly after awakening. Participants were asked to record the time at which each saliva sample was taken. They were instructed to store their saliva in their freezers until the day of the visit. At the lab, the saliva samples were centrifuged at 3000 rpm for 10 minutes and then stored at -20^{o}C until assayed. The cortisol in saliva was measured without extraction using an in house competitive radio-immunoassay employing a polyclonal anticortisol-antibody (K7348). [1,2-^{3}H(N)]-Hydrocortisone (NET185, NEN - DUPONT, Dreiech, Germany) was used as a tracer. The lower limit of detection was 0.5 nmol/L and inter-assay variation was 9% at 3 nmol/L and 5% at 23 nmol/L. Intra-assay variation was 4%.

The cortisol awakening response was assessed by calculating the area under the curve to the ground (AUCg). We also calculated the area under the curve with respect to increase (AUCi), but since this measure did not result in different findings and was highly correlated to AUCg (r=0.55), we only used the AUCg. Second, resting levels of cortisol were defined as the average of the saliva samples taken at 10PM and 11PM. Third, as an indicator of suppression of the HPA-axis, the cortisol value was taken at awakening the morning after the ingestion of the dexamethasone. Because evening cortisol levels and awakening cortisol after the dexamethasone suppression test (DST) were skewed these data were natural log-transformed.

Other variables

Educational level was divided into eight categories, graded from primary school to academic degree, according to the Dutch educational system. During the patient's

visit to the medical center, an overnight fasting venous blood sample was taken to determine glucose levels. Height and weight were measured without shoes and heavy clothing, and the body mass index (BMI) was calculated (kg/m^2). Systolic and diastolic blood pressures (mm Hg) were measured three times with a sphygmomanometer and averaged. Diabetes mellitus was defined as a history of diabetes mellitus, glucose ≥7.0 mmol/L or self reported use of oral antidiabetic drugs or insulin. Smoking habits and alcohol intake were assessed with questionnaires. Pack-years of smoking was calculated and alcohol use was categorized into <1 drink p/week, 1-20 drinks p/week, and >20 drinks p/week. Depressive symptoms in the past two weeks were assessed using the Patient Health Questionnaire-9 (PHQ-9).[27].

Data-analysis

Missing data rarely occur at random and a complete case analysis (deletion of all participants with one or more missing values) leads to loss of statistical power and to biased results. We therefore used multiple imputation (10 datasets) to address the missing values[28;29] using the statistical programme R (version 2.10.0; areg Impute). Data were analyzed using SPSS version 17.0 (Chicago, Ill, USA), by pooling the 10 imputed datasets.

Life events and cognitive functioning

Generalized linear models adjusted for age, sex, level of education, systolic and diastolic blood pressure, diabetes, BMI, drinking and smoking habits, and depressive symptoms were used to estimate the cross-sectional associations of childhood abuse, childhood events and recent events with z-scores of cognitive functioning. For each of the three cognitive domains (memory; executive functioning; processing speed and attention) separate models were made, with childhood abuse (yes vs. no), childhood events (yes vs. no) and recent events (no events; one event; two or more events) entered as independent variables. Furthermore, because most of the participants with a history of childhood events (64 %) and abuse (69 %) also reported recent events, analyses on childhood events and abuse were adjusted for recent events, and analyses on recent events were adjusted for childhood events and abuse.

To examine whether recent life events modulated the association of childhood events/abuse with cognitive functioning, we added interaction terms between recent life events and childhood abuse and between recent life events and childhood events.

Role of HPA-axis activity

Using general linear models we estimated adjusted mean differences in cortisol levels according to the life event categories. Secondly, we estimated adjusted mean differences in z-scores of cognitive domains according to tertiles of cortisol measures (AUCg; evening cortisol and awakening cortisol after DST). Thirdly, to examine whether the relation between life events and cognition was explained or mediated by

cortisol measures we estimated z-scores of cognitive functioning by entering cortisol measures and life events variables both as independent variables in the model. All associations were adjusted for age, sex, level of education, systolic and diastolic blood pressure, diabetes, BMI, drinking and smoking habits, depressive symptoms and time of awakening.

3. Results

The mean age of the sample was 62±9 years and the majority was male (83%). Childhood abuse was reported by 20% and childhood events by 51% of the population; 34% reported one and 23% two or more recent events (Table 1). The most often cited childhood events were emotional neglect, paternal and familial discourse at home and the experience of a disaster or severe accident. The most often cited recent events were severe illness, injury or assault to one self and illness or death of a close relative (Table 2).

Table 1. Baseline characteristics of the study sample

N	736	Missing data %
Male (%)	82	0
Age[a]	62 (9)	0
Level of education (0-8)[a,c]	4 (2)	1
Body mass index[a]	27 (4)	0.5
Packyears smoking[b]	19 (0 – 49)	3
Diabetes (%)	21	2
Blood pressure Hg/mm		1
- Diastolic	82 (11)	
- Systolic	143 (19)	
Alcohol consumption (%)		0
- <1 drink/week	31	
- 1-20 drinks/week	58	
- >20 drinks/week	11	
Childhood abuse (%)	20	0.5
Childhood events (%)	51	0.5
Recent events (%)		10
- no event	39	
- 1 event	33	
- 2 or more events	28	
Depressive symptoms (0-27)[b]	2.0 (0.0 – 8.0)	1
Cognition		
MMSE (0 – 30)[b]	29 (27 – 30)	0
Recall Rey complex figure test (0 – 36)[a]	20.4 (6.4)	2
Verbal memory[a]		1
- Immediate recall (0 – 75)	38.7 (10.6)	
- Delayed recall (0 – 15)	7.8 (3.2)	
Visual elevator timing score (0 -10)[b]	4.4 (3.0 – 7.6)	3
Brixton spatial anticipation test (0 -10)[b]	6.0 (2.0 – 7.0)	2
Verbal fluency[b]	10.0 (6.0 – 17.0)	0.1
Digit span[a]		0.4
- Forward (0 – 16)	8.3 (1.9)	
- Backward (0 – 14)	5.6 (1.9)	
Digit Symbol Substitution Test[a]	55.0 (16.0)	2
HPA-axis activity		
AUCg morning cortisol (nmol/L*hour)[a]	17.0 (6.6)	17
Evening cortisol (nmol/L)[b]	3.4 (2.0 – 6.3)	11
Awakening cortisol after DST (nmol/L)[b]	1.6 (0.8 – 3.5)	18

[a] data presented as mean (sd)
[b] data presented as median (interdecile range 10 – 90 %)
[c] education level ranges from 1 (primary school) to 8 (university)
AUCg: area under the curve to the ground; DST: dexamethasone suppression test

Table 2. Frequencies of childhood abuse, childhood events, and recent events

	N (%)
Total number childhood abuse	211
Emotional neglect	78 (37)
Psychological abuse	52 (25)
Physical abuse	47 (22)
Sexual abuse	34 (16)
Total number of childhood events	502
Death of mother	23 (5)
Death of father	50 (10)
Death of sibling	56 (11)
Maternal deprivation	58 (11)
Paternal discourse	75 (15)
Familial discourse	72 (15)
Divorce parents	22 (4)
Disaster or accident	146 (29)
Total number recent events	763
Severe illness, injury or assault	85 (11)
Death of partner/parent/child	50 (7)
Death of other relative	222 (29)
Severe illness of relative/loved one	135 (17)
Relational break-up	14 (2)
Divorce and marital problems	15 (2)
Severe conflict with friends	47 (6)
Long-term unemployment	28 (4)
Discharged from job	21 (3)
Severe financial problems	35 (4)
Breaking the law	14 (2)
Victim of crime	21 (3)
Other	76 (10)

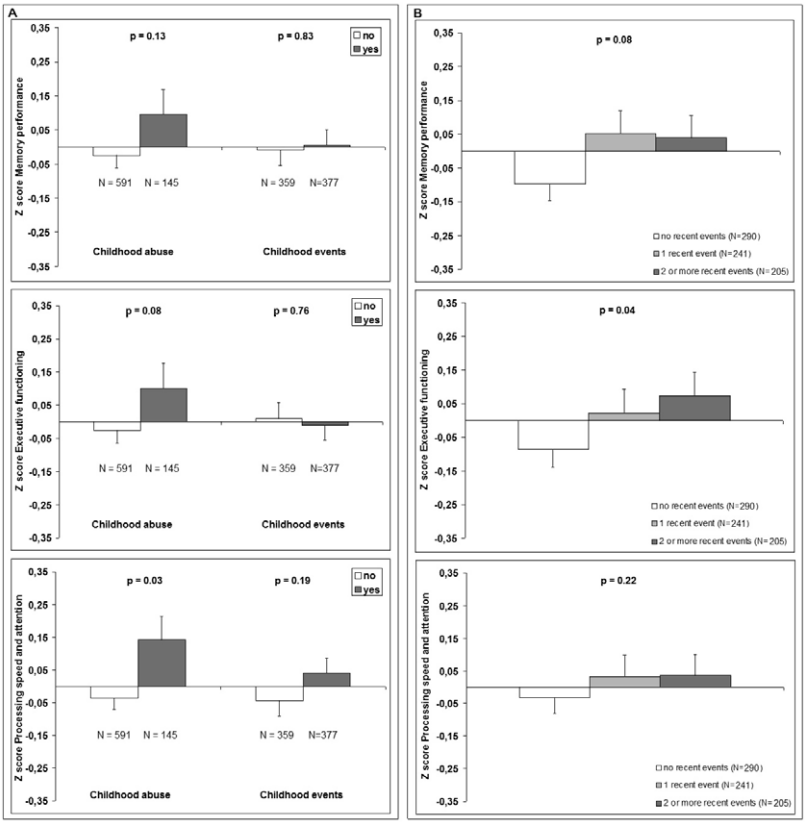
Life events and cognitive functioning

Participants who reported a history of childhood abuse had significantly better memory performance (mean difference in z-score: 0.16; 95% CI 0.00 to 0.32; p=0.05) and better processing speed and attention (mean difference in z-score: 0.18; 95% CI 0.03 to 0.34; p=0.02), and better executive functioning, although not significantly (mean difference in z-score: 0.13; 95% CI -0.04 to 0.30 p=0.13). The experience of childhood events was not associated with any of the cognitive domains (Figure 1a).

Participants with more recent events had better executive functioning (compared to no events, mean difference in z-score for one recent event: 0.16; 95% CI 0.00 to 0.32;

p=0.06, and for two or more recent events: 0.18; 95% CI 0.02 to 0.35; p=0.04) and borderline significantly better memory performance (compared to no events, mean difference in z-score for one recent event: 0.16; 95% CI 0.01 to 0.31; p=0.04, and for two or more recent events: 0.10; 95% CI -0.07 to 0.27; p=0.23), but not statistically significant better processing speed and attention (compared to no events, mean difference in z-score for one recent event: 0.08; 95% CI -0.09 to 0.24; p=0.36, and for two or more recent events: 0.08; 95% CI -0.08 to 0.23; p=0.32) (Figure 1b).
The interaction terms between childhood abuse and recent life events were not significant on any of the cognitive domains (p for interaction term >0.05).

Figure 1. Adjusted mean Z-scores of memory functioning, executive functioning and processing speed and attention, according to childhood abuse and childhood events (A), and number of recent life events (B)



Z-scores are adjusted for age, sex, level of education, systolic and diastolic blood pressure, diabetes mellitus, body mass index, pack years of smoking, alcohol consumption, depressive symptoms and childhood events/ abuse or recent events.
Error bars represent standard errors.
P-values are for trend of main effect.

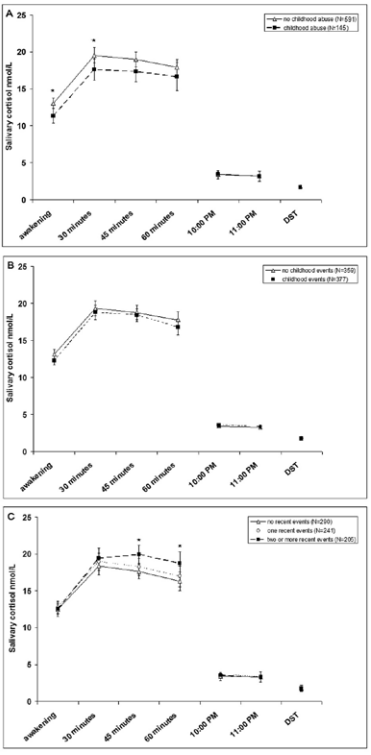
Role of HPA-axis activity

Participants who reported childhood abuse had lower AUCg of morning cortisol (mean difference -1.56 nmol/L*hour; 95% CI -2.83 to -0.30; p=0.047), but evening cortisol and awakening cortisol after DST were not different from those reporting no abuse (Figure 2a). The experience of childhood events was not associated with any of the cortisol measures (Figure 2b).

Patients with two or more recent events had significantly higher AUCg of morning cortisol than those without recent events (B=1.31 nmol/L*hour; 95% CI 0.01 to 2.60; p=0.048). No associations were found with evening cortisol or awakening cortisol after DST (Figure 2c).

None of the cortisol measures were significantly associated with cognitive functioning (table 3). Consequently, when cortisol measures were added to the models with life events as independent variables and z-socres of cognition as dependent variables, none of the associations altered.

Figure 2. Adjusted salivary cortisol levels according to childhood abuse (A), childhood events (B) and recent life events (C).



Error bars represent 95 % confidence intervals; *p < 0.05

Mean levels are adjusted for age, sex, systolic and diastolic blood pressure, diabetes mellitus, body mass index, pack years of smoking, alcohol consumption, time of awakening, and depressive symptoms.

Table 3. Associations between tertiles of cortisol measures and Z-scores of cognitive domains

	Memory performance B (95 % CI)	**Executive functioning B (95 % CI)**	**Processing speed and attention B (95 % CI)**
AUCg morning cortisol			
≤ 13,89 nmol/L	Ref	Ref	Ref
13,9 – 19,11 nmol/L	-0.02 (-0.19 to 0.15)	-0.06 (-0.23 to 0.11)	0.04 (-0.11 to 0.20)
≥ 19,12 nmol/L	-0.09 (-0.26 to 0.08)	-0.17 (-0.35 to 0.12)	-0.08 (-0.24 to 0.08)
Evening cortisol			
≤ 2.80 nmol/L	Ref	Ref	Ref
2.81 – 4.05 nmol/L	-0.01 (-0.18 to 0.15)	-0.05 (-0.21 to 0.12)	-0.07 (-0.24 to 0.10)
≥ 4.06 nmol/L	-0.06 (-0.21 to 0.10)	-0.05 (-0.21 to 0.12)	-0.01 (-0.18 to 0.15)
Awakening cortisol after DST			
≤ 1.4 nmol/L	Ref	Ref	Ref
1.41 – 2.0 nmol/L	-0.07 (-0.24 to 0.11)	-0.03 (-0.20 to 0.13)	0.00 (-0.18 to 0.17)
≥ 2.01 nmol/L	-0.11 (-0.29 to 0.06)	-0.08 (-0.25 to 0.09)	-0.12 (-0.29 to 0.04)

Z-scores are adjusted for age, sex, systolic and diastolic blood pressure, diabetes mellitus, body mass index, pack years of smoking, alcohol consumption, time of awakening, and depressive symptoms.

4. Discussion

In this large cohort study among participants with a history of atherosclerotic disease, childhood abuse was associated with better cognitive functioning, while childhood events, were not associated with cognitive functioning. Recent life events was associated with better executive functioning. These associations were not explained by HPA-axis activity, as HPA-axis activity was not associated with cognitive functioning.

Two preceding observational studies also found better cognitive functioning with recent stressors. One study found that recent negative life events were associated with better overall cognitive performance,[8] while another study observed better memory performance during periods of exam stress.[14] It could be hypothesized that the experience of recent events in our study resulted in heightened arousal, which may have resulted in improved executive functioning, as has also been suggested in relation to anxiety.[30] However, poorer memory performance,[31] executive functioning[7] and processing speed and attention have also been reported,[7] making this explanation not straightforward. However, studies are not fully comparable, as older persons

were used in some studies. Also, in the longitudinal study by Peavy and colleagues, a detrimental effect of psychosocial stress on cognition was only found in persons who already had mild cognitive impairment and not in cognitively intact persons. [3]

Unexpectedly, we also found that childhood abuse was associated with better cognitive functioning. A beneficial effect of a history of childhood abuse on neuropsychiatric outcomes is contrary to general beliefs in psychiatry research, and childhood abuse has been associated with poorer memory performance.[4;6] However, it has been suggested that childhood adversities do not necessarily result in poor outcome later in life and that resilience after childhood adversities is quite common (for review see:[32]).

A tentative explanation for the better performance in persons with a history of childhood abuse comes from animal studies. Recently, it has been described that rodents who had received poor maternal care could cope better with stressful situations later in life than rodents who had received good maternal care.[33;34] Furthermore, rodents with poor maternal care showed better memory performances during stressful circumstances than rodents who had received good maternal care. Since cognitive testing is considered to be a stressful experience,[35] it could be hypothesized that persons with severe stress (i.e. abuse) during childhood perform better during the stressful circumstance of neuropsychological testing.

Contrary to childhood abuse, we found no association of childhood events with cognitive functioning and also not with HPA-axis activity. Perhaps the childhood events were less severe stressors than childhood abuse and may have therefore not affected cognitive functioning later in life. It should be noted though that the effect estimates for childhood abuse were rather small although they were statistically significant.

Stressful life events can lead to long-term changes in HPA-axis activity and it has frequently been suggested that this could result in cognitive impairment and even cognitive decline. In line with previous findings from our study group in a different population as well as others [9;36] we found that childhood abuse was associated with lower morning cortisol levels, whereas recent life events were associated with higher morning cortisol levels. Nevertheless, we found no support for glucocorticoid mediated cognitive impairment. Few studies investigated the mediating role of HPA-axis activity in the relation between stressful experiences and cognitive functioning, with one study also not finding support for a mediating role of the HPA-axis [3] while another study found that stress during exam periods was associated with lower cortisol levels and better memory performance, but poorer attention. Cortisol levels were not correlated with cognitive functioning in this study, so it is uncertain whether cortisol levels mediated this relation.[14]

Our study is among the first to investigate the associations of stressors during childhood and stressors later in life with cognitive functioning and the role of HPA-axis activity in this relation. The study sample was large and cognitive functioning was

assessed by an extensive neuropsychological battery, making it possible to investigate three cognitive domains. Another strength is that HPA-axis activity was measured using seven cortisol samples and therefore we could examine the diurnal variability and dexamethasone suppression test and examine in great detail how HPA-axis activity plays a role in the relation between life events and cognitive functioning.

Our study had a cross-sectional design and it could be argued that persons who reported life events had better cognitive functioning before the life events occurred. However, if so one would expect to have found a relation between childhood events and cognition as well. Also, the overall level of cognitive functioning as measured with the Mini Mental State Examination was high, making recall bias less likely.

The cross-sectional design could also explain why we found no relation between cortisol levels and cognitive functioning, as it has been suggested that particularly change in basal cortisol levels over time is a risk factor for cognitive decline. However, it is also possible that the relatively young age of our population made it more difficult to find significant associations. Previous studies particularly found associations between cortisol and cognition in older persons [37-39] and in persons with MCI.[40]

Life events were assessed by asking whether they had occurred or not, and this method does not take into account whether the reported events were also experienced as being stressful or traumatic. It is thus possible that life events are only detrimental to the brain if they are also experienced as being stressful or traumatic.

Our study population consisted of patients with a history of atherosclerotic disease. We thus do not know if the observed associations can be generalized to the general population. However, we expected that the relation between life events, HPA-axis activity and cognitive functioning in a population with high vascular burden would make it more likely to find an association that would not have been observed in younger and healthier populations.

In conclusion, in this study childhood abuse and to a lesser extent recent life events were associated with better cognitive functioning. Basal HPA-axis activity was not associated with cognitive functioning, and could therefore not explain the relation between stressors and cognitive functioning. Further studies should also investigate psychosocial stressors, HPA-axis activity, and cognition in combination to unravel the relation between stress and cognition.

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Chapter 4

Depression, HPA-axis and the brain

4

4.1 Basal hypothalamic pituitary adrenal axis activity and hippocampal volumes. The SMART-Medea study

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Abstract

Background

It has frequently been hypothesized that high levels of glucocorticoids have deleterious effects on the hippocampus and increase risk for cognitive decline and dementia, but no large-scale studies in humans examined the direct relation between hippocampal volumes and hypothalamic-pituitary-adrenal (HPA) axis activity.

Methods

Cross-sectional analyses within the SMART-Medea study, an ancillary study to the SMART-MR study on brain changes on MRI among patients with arterial disease. In 575 patients (mean age 62±9 years) diurnal cortisol rhythm was assessed with 6 saliva samples, collected at awakening and 30, 45 and 60 minutes thereafter, and at 10PM and 11PM. A low dose of dexamethasone (0.5 mg) was administered at 11PM and saliva was sampled the next morning at awakening. Volumetric measurements of the hippocampus were performed on a 3-dimensional FFE T1-weighted MRI scan with isotropic voxels.

Results

Mean total relative hippocampal volume was 6.0±0.7 ml. Linear regression analyses, adjusted for age, sex, vascular risk factors and global brain atrophy showed that participants with higher evening levels and higher awakening levels after dexamethasone had smaller hippocampal volumes (B per SD (4.2) increase= -0.09 ml; 95%CI -0.15 to -0.03 ml and B per SD (2.5) increase = -0.07 ml; 95%CI -0.13 to -0.01 ml, respectively). The awakening response was not significantly associated with hippocampal volumes.

Conclusion

In this population, higher evening cortisol levels and reduced suppression after dexamethasone were associated with smaller hippocampal volumes, independent of total brain volume. The cortisol response after awaking was not associated with hippocampal volume.

1. Introduction

The hippocampus, located in the medial temporal lobe, is important for memory functioning and one of the first structures to be affected in Alzheimer's disease. [1,2] It has been hypothesized that alterations in hypothalamic-pituitary-adrenal (HPA) axis activity, especially hypersecretion of glucocorticoids, have adverse effects on hippocampal neurons. [3-5] The HPA-axis is an important neuroendocrine system involved in responses to stress. Hippocampal neurons are not only a target for glucocorticoids but also play an important role in regulating the HPA-axis activity by inhibiting glucocorticoid secretion. [6] According to the glucocorticoid cascade hypothesis [7], elevated levels of cortisol, an important glucocorticoid in humans, lead to damage to hippocampal neurons, which, in turn, will result in diminished inhibition of glucocorticoids. This diminished inhibition will then result in higher levels of cortisol and lead to further damage and neuronal loss of the hippocampus. [7] Support for this hypothesis comes predominantly from animal studies where overexposure of glucocorticoids leads to remodelling and atrophy of hippocampal neurons in rodents and non-human primates. [7-9]

Evidence from human research supporting this hypothesis is mainly indirect. A decrease in hippocampal volume has been observed in patients with elevated cortisol levels due to Cushing's disease (4) and in corticosteroid dependent patients with asthma or rheumatic illness. [10] However, the effect of cortisol on hippocampal volume may be reversible. [11] One study showed increase of hippocampal volumes after a decrease of the steroid exposure as a result of successful treatment of Cushing's disease. [12] Also, many studies, although not all, [13,14] reported smaller hippocampal volumes in patients with stress-related disorders, such as post-traumatic stress disorder (PTSD) or major depressive disorder. [15-18] Fewer studies investigated the direct association between basal HPA-axis activity and hippocampal volume in humans, and findings are inconsistent. Some studies observed that prolonged elevated cortisol levels were associated with smaller hippocampal volume in older healthy subjects . [3,19,30] However, others found no relation between HPA-axis activity and hippocampal volume. [21-24]

With the exception of one study among depressed patients that found no relationship between cortisol levels and hippocampus size, [24] the existing studies were based on small sample sizes or were done in young or healthy elderly subjects. Selecting healthy subjects may reduce the ability to detect an association between cortisol levels and hippocampal volumes, because one of the reasons subjects stayed healthy may be that there is no disturbance in HPA-axis functioning. [25] Since alterations in HPA activity have been described in patients with diabetes, [26] hypertension, [27] and the metabolic syndrome, [28] an association between HPA-axis activity and hippocampal volume may be more easily detected in a population with a high burden of vascular risk.

This study examined the association between basal HPA-axis activity and hippocampal volume in a large population of subjects with arterial disease.

2. Methods

Subjects

Data were used from the Second Manifestations of ARTerial disease-Magnetic Resonance (SMART-MR) study, a prospective cohort study aimed to investigate brain changes on MRI in 1309 independently living patients with symptomatic atherosclerotic disease. Details of the design and participants have been described elsewhere. [29,30] In brief, between May 2001 and December 2005, all patients newly referred to the University Medical Center Utrecht with manifest coronary artery disease, cerebrovascular disease, peripheral arterial disease or an abdominal aortic aneurysm (AAA), and without MR contraindications were invited to participate. During a 1-day visit to our medical center, an MRI of the brain was performed, a physical examination, and blood and urine sampling. Risk factors, medical history, and functioning were assessed with questionnaires.

Between January 2006 and May 2009, all participants still alive were invited for follow-up measurements, including MRI of the brain, neuropsychological testing, a physical examination, blood and urine sampling, risk factors, medical history, and functioning. In addition, as part of the SMART-Medea (Memory, depression and aging) study, an ancillary study to the SMART-MR study, aimed to investigate brain changes associated with psychosocial vulnerability and stress factors, measurements of salivary cortisol and psychosocial stressors early and later in life were added. From March 2006, diagnostic assessment of depression was added and a T1-weighted 3-dimensional fast field-echo sequence for measuring hippocampal volumes. The SMART-MR and SMART-Medea study were approved by the ethics committee of our institution and written informed consent was obtained from all participants. In total, 754 of the surviving cohort (61% of n=1,238) gave written informed consent; 466 (38%) persons refused and 18 (1%) were lost to follow-up.

HPA-axis activity

HPA-axis activity was assessed at home by 7 measurements of cortisol in saliva over a period of 24 hours. Cortisol exists in free (non-protein-bound) form in saliva. The free form is the biologically active one. [31] The saliva was collected using cotton dental rolls (Salivette, Startstedt). Participants were instructed to refrain from smoking, drinking caffeine, eating or cleaning their teeth at least 30 minutes before collecting a saliva sample and were asked to chew on the rolls for at least 2 minutes. On day 1, participants were instructed to take the first sample immediately after awakening while still lying in bed, and to take the second, third and fourth samples after 30, 45 and 60 minutes. Sample 5 and 6 were collected at 10 PM and 11 PM. Furthermore, participants were asked to take 0.5 mg of dexamethasone orally after their sixth saliva sample, and to sample their saliva the next morning directly after awakening. They were instructed to sample on a normal weekday and to store their saliva in their freezers until the day

of the visit. At the lab, the saliva samples were centrifuged at 3000 rpm for 10 minutes and then stored at -20ºC until assayed. The cortisol in saliva was measured without extraction using an in house competitive radio-immunoassay employing a polyclonal anticortisol-antibody (K7348). [1,2-^{3}H(N)]-Hydrocortisone (NET185, NEN-DUPONT, Dreiech, Germany) was used as a tracer. The lower limit of detection was 0.5 nmol/L and inter-assay variation was 9% at 3 nmol/L and 5% at 23 nmol/L. Intra-assay variation was 4%.

HPA-axis activity and its diurnal pattern were quantified in several ways. First, the cortisol awakening response, which is the immediate increase of cortisol in the hour after awakening, was computed as the area under the cortisol curve with respect to zero (AUC). [32] The AUC was calculated by multiplying the levels of the four morning cortisol samples by the time interval between sampling points in minutes. In addition, the rise was defined as the difference between the sample direct after awaking and the sample taken at 30 minutes. [33,34] Second, resting evening levels of cortisol were defined as the average of the saliva samples taken at 10PM and 11PM. Third, as an indicator of suppression of the HPA-axis, the cortisol value was taken at awakening the morning after the ingestion of the dexamethasone.

Magnetic Resonance Protocol

The MR investigations were performed on a 1.5-T whole-body system (Gyroscan ACS-NT, Philips Medical Systems, Netherlands). The protocol consisted of a transversal T1-weighted gradient-echo sequence (repetition time (TR)/echo time (TE): 235/2 ms; flip angle, 80º), a transversal T2-weighted turbo spin-echo sequence (TR/TE: 2200/11 ms and 2200/100 ms; turbo factor 12), a transversal T2-weighted fluid attenuating inverse recovery (FLAIR) sequence (TR/TE/inversion time (TI): 6000/100/2000 ms) and a transversal inversion recovery (IR) sequence (TR/TE/TI: 2900/22/410 ms) (field of view (FOV) 230x230 mm; matrix size 180×256; slice thickness 4.0 mm; no gap; 38 slices). Furthermore, for measurements of the hippocampal volume, we obtained a sagittal T1-weighted 3D FFE (fast field echo) sequence (TR/TE: 7.0/3.2 ms; flip angle, 8º), FOV 240 mm; matrix size, 240×256; slice thickness 1.0 mm; no gap; 170 slices).

Brain segmentation

We used the T1-weighted gradient-echo, IR sequence, and FLAIR sequence for brain segmentation. The probabilistic segmentation technique according to the k-nearest neigbor (KNN) classification has been described elsewhere. [35] The results of the segmentation analysis were visually checked for the presence of infarcts and adapted if necessary to make a distinction between WML and infarct volumes. Total brain volume was calculated by summing the volumes of gray and white matter and, if present, white matter lesions and infarcts. Total intracranial volume was calculated by summing total brain volume and volumes of sulcal and ventricular cerebrospinal fluid. Total brain volume was divided by intracranial volume to obtain brain parenchymal

fraction (BPF), an indicator for global atrophy. All volumes cranial to the foramen magnum were included in the segmentation results. Thus, the total brain volume includes the cerebrum, brainstem and cerebellum.

Assessment of hippocampal volume

The sagittal T1-weighted images were tilted to the coronal plane and orientated perpendicular to the long axis of the left hippocampus. The hippocampus was manually outlined on an average of 40 slices and included the hippocampus proper, subiculum, fimbria, alveus, and dentate gyrus (Figure 1).The most anterior slice was defined as the slice where the hippocampus appeared below the amygdala. The alveus, which was clearly visible, formed the dorsal border of the hippocampus on the anterior slices and was used to separate the hippocampal head from the amygdala. More posterior, the dorsal boundary was defined by CSF and choroid plexus, which was not included in the measurements. The posterior border was defined as the slice where the total length of the fornix was visible. The lateral boundaries were defined by CSF of the temporal horn of the lateral ventricle and by the gray-white matter border of the temporal stem. Medially, the hippocampus was bounded by CSF in the cisterna ambiens and transverse fissure, and ventrally by white matter of the parahippocampal gyrus

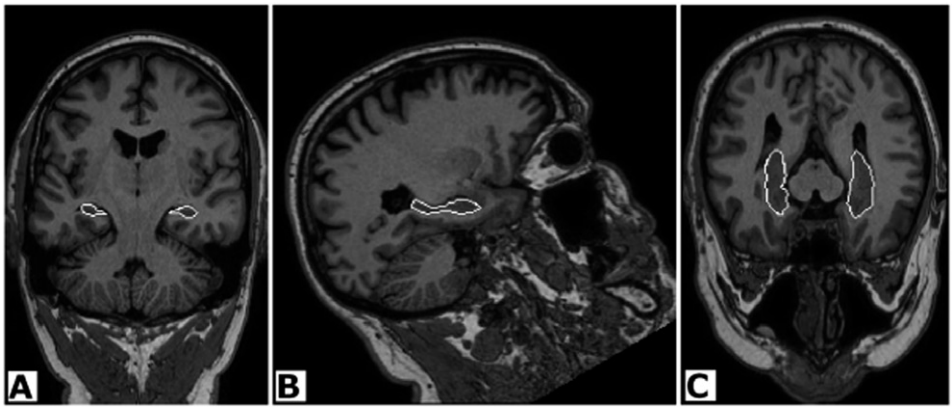
Measurements of hippocampal volumes were performed by two trained investigators, blinded to all clinical information. Left and right hippocampal volumes were calculated by multiplying the total number of voxels by the volume of a voxel (1.0x0.94x0.94 mm). The intra-rater reliability coefficient for repeated tracing in 20 randomly selected hippocampi was 0.96 and 0.98, and the inter-rater agreement was 0.96. The coefficient of variation for the two raters was 3.8 %.,[36] and the mean difference between raters was 11.1 voxels.

Covariates

Participants were asked to record saliva sampling time and to complete a questionnaire on current use of medication, smoking habits and alcohol use. During the patient's visit an overnight fasting blood sample was drawn to determine glucose and lipid levels. Height and weight were measured and body mass index (BMI) (kg/m^2) was calculated. Systolic and diastolic blood pressures (mmHg) were measured with a sphygmomanometer three times while the patient was seated and the average of the measurements was calculated. Diabetes mellitus was defined as a glucose level of ≥7.0 mmol/l or use of oral antidiabetic drugs or insulin at baseline or follow-up. Hyperlipidemia was defined as total cholesterol >5.0 mmol/l, low-density lipoprotein cholesterol >3.2 mmol/l or use of lipid lowering drugs. Twelve-month prevalence of major depressive disorder according to DSM-IV criteria was assessed using the Composite International Depression Interview (CIDI). [37] General cognitive functioning was assessed with the Mini Mental State Examination (MMSE)[38] and participants with

a score <27 were examined by a physician who conducted an additional in-house standardized dementia interview and physical examination. The results of these investigations together with the outcome of the neuropsychological testing were discussed in a consensus meeting with a geriatrician to diagnose dementia.

Figure 1. Magnetic resonance image of the hippocampal formation in three orientations.



Shown are coronal (panel A), sagittal (panel B) and axial (panel C) magnetic resonance images. The hippocampus is manually outlined (white line).

Study sample

Of the 754 persons who participated, 709 patients received an MRI scan. Nineteen scans had artefacts and because the 3D T1-weighted FFE sequence was added from March 14, data for hippocampal volumes were missing in 54 patients. Of the remaining 636 subjects, the data of cortisol levels of 61 patients were missing, due to development of the sampling protocol, refusal of patients to collect saliva, non-adherence to the sampling times and because some of the samples were misplaced. These subjects did not differ in mean hippocampal volume from the study sample (5.9±0.7 ml vs. 6.0±0.7 ml). These 61 subjects were also excluded, leaving 575 subjects for analysis.

Data analysis

Crude hippocampal volumes were divided by ICV and multiplied by the mean ICV of the study sample (1454 ml) to obtain relative hippocampal volumes in ml. We summed the left and right sides to yield total volumes because the analyses did not suggest laterality of effects. The association between HPA-axis activity and hippocampal volume was assessed with analysis of variance (ANOVA) and multiple linear regression analysis.

First, we calculated baseline characteristics for the total study sample and across tertiles of evening cortisol. Second, we used linear regression analysis to

estimate the age-adjusted associations between vascular risk factors and hippocampal volumes. Third, Pearson's product moment correlation coefficients between cortisol measures were calculated. Fourth, we calculated mean relative hippocampal volumes according to tertiles of the rise, AUC, evening cortisol and cortisol suppression after dexamethasone. Post-hoc between-group testing was performed using the Fisher LSD test. Furthermore, linear regression analysis was used to estimate the associations per standard deviation (SD) increase of the rise, AUC, evening level and cortisol suppression after dexamethasone with mean relative hippocampal volumes. In the first model we adjusted for age and sex. In the second model, additional adjustments were made for systolic and diastolic blood pressure, diabetes mellitus (yes vs. no), hyperlipidemia (yes vs. no), pack-years of smoking, alcohol use (<1 units, ≥1 units ≤20, >20 units per week), BMI, and history of disease (coronary artery disease, cerebrovascular disease, peripheral arterial disease, or AAA). In the third model, we additionally adjusted for BPF to investigate whether the association between HPA-axis activity and hippocampal volume was independent of global brain atrophy. We repeated all analyses after excluding patients with major depressive disorder or with a diagnosis of dementia.

3. Results

The mean age ± SD of the study sample was 62±9 years, the majority were men (81%), and the majority had a history of coronary artery disease (62%). The mean relative total hippocampal volume was 6.0±0.7 ml (Table 1). Table 2 shows the age-adjusted association between vascular risk factors and hippocampal volume. There was a strong positive correlation between evening levels and cortisol levels after dexamethasone suppression and between the AUC and the rise. Also, there was a positive correlation between the AUC and evening levels, and between the AUC and awakening levels after dexamethasone (Table 3).

Table 1. Characteristics of the study sample (n=575) across tertiles of evening cortisol levels

Evening cortisol levels	*Lower tertile <2.8 nmol/l*	*Middle tertile 2.8-4.1 nmol/l*	*Upper tertile >4.1 nmol/l*	*Total*
Age (years)*	60 ± 9	62 ± 9	64 ± 9	62 ± 9
Sex (male)†	78	83	82	81
Blood pressure (mmHg)*				
Diastolic	82 ± 10	83 ± 11	80 ± 11	82 ± 11
Systolic	142 ± 16	144 ± 21	141 ± 19	142 ± 18
Diabetes mellitus†	21	19	25	22
Hyperlipidemia†	83	82	82	83
Body mass index (kg/m^2)*	28 ± 4	27 ± 4	27 ± 4	27 ± 4
Smoking (pack-years)‡	18 (0, 49)	20 (0, 53)	21 (0, 51)	19 (0, 50)
Alcohol consumption†				
< 1 units/week	37	27	27	31
1 - 20 units/week	53	61	58	58
> 20 units/week	9	12	16	12
Disease history†				
Coronary artery disease	67	64	64	62
Cerebrovascular disease	18	24	25	24
Peripheral arterial disease	14	17	25	18
Abdominal aortic aneurysm	8	5	6	6
Major depressive disorder†	5	8	9	7
Cortisol (nmol/l)*				
Rise	6.4 ± 8.4	7.0 ± 7.2	6.4 ± 7.6	6.6 ± 7.8
AUC	15.7 ± 6.9	17.9 ± 6.0	18.2 ± 7.4	17.2 ± 6.9
After dexamethasone	1.6 ± 1.6	1.9 ± 1.2	3.2 ± 3.8	2.2 ± 2.5
Total hippocampal volume (ml)*	6.1 ± 0.6	5.9 ± 0.7	5.8 ± 0.4	6.0 ± 0.7
Brain parenchymal fraction†	79 ± 3	78 ± 3	78 ± 3	78 ± 3
Intra cranial volume (ml)*	1449 ± 130	1467 ± 136	1443 ± 125	1454 ± 131

*mean ± SD, † percentage, ‡ median (10th percentile, 90th percentile)

Table 2. Age adjusted associations of vascular risk factors with hippocampal volumes.

	Hippocampal volume/ICV		
	B	**(95% confidence interval)**	**P value**
Age (years)	-0.011	(-0.017 to -0.05)	<0.001
Men vs. women	0.198	(0.052 to 0.344)	0.008
Systolic blood pressure	0.003	(-0.001 to 0.006)	0.111
Diastolic blood pressure	0.004	(-0.001 to 0.010)	0.107
Diabetes mellitus (yes vs. no)	0.042	(-0.100 to 0.183)	0.579
Hyperlipidemia (yes vs. no)	0.048	(-0.105 to 0.201)	0.593
Body mass index (kg/m^2)	0.011	(-0.004 to 0.026)	0.137
Smoking (pack years)	-0.001	(-0.004 to 0.002)	0.426
Alcohol consumption	-0.030	(-0.126 to 0.059)	0.473

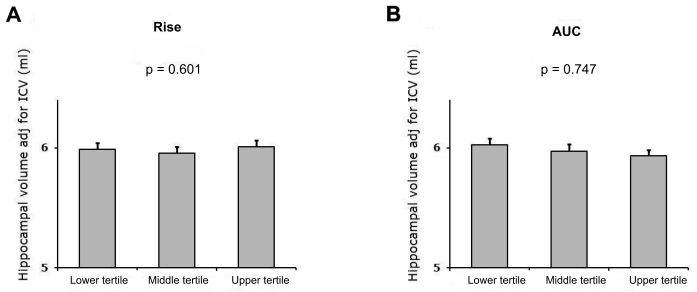
Table 3. Pearson's correlations between the cortisol measurements

	Rise	*AUC*	*Evening level*	*After dexamethasone*
Rise	-	0.601*	0.014	-0.004
AUC	0.601*	-	0.237*	0.192*
Evening level	0.014	0.237*	-	0.631*
After dexamethasone	-0.004	0.192*	0.631*	-

* p< 0.05

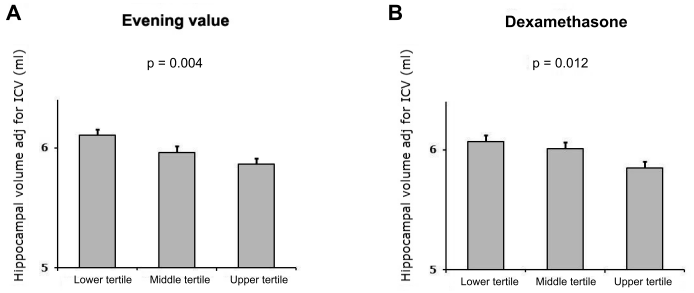
There were no significant differences in hippocampal volumes between tertiles of the rise or AUC (Figure 2). However, subjects with higher levels of evening cortisol had smaller hippocampal volumes (F=5.56; p=0.004) (Figure 3A). The mean difference between the upper and the lower tertile of evening cortisol levels was -0.24 ml (95% CI -0.384 to -0.098 ml, p=0.001). There was also a significant difference in hippocampal volume between tertiles of awakening cortisol after dexamethasone (F=4.48; p=0.012) (mean difference between upper and lower tertile 0.22 ml; 95% CI -0.369 to -0.072) (Figure 3B).

Figure 2. Hippocampal volume relative to intracranial volume (SEs) according to tertiles of the awakening response, quantified as the rise (tertiles in nmol/l < 2.5, 2.5 – 9.2 and >9.2) (A) and AUC (tertiles in nmol/l < 14.0, 14.0 -18.4 and >18.4) (B).



Analysis of variance revealed no differences between groups effect for the rise (F=0.29, p=0.75) and the AUC (F= 0.51, p= 0.60)

Figure 3. Hippocampal volumes relative to intracranial volume (SEs) according to tertiles of cortisol levels in the evening (tertiles in nmol/l <2.8, 2.8 - 4.1 and > 4.1) (A) and after dexamethasone (tertiles in nmol/l <1.4, 1.4 – 2.0 and >2.0) (B).



Analysis of variance revealed significant differences between groups effect for the cortisol levels in the evening (F= 5.6; p= 0.004), and for cortisol levels after dexamethasone (F= 4.8, p= 0.012).

Using linear regression analyses, again no association was found between the AUC or rise and hippocampal volumes after adjustment for age and sex or other covariates (Table 4). After adjusting for age and sex, total hippocampal volume still decreased with increasing evening cortisol levels (B= -0.10 ml; 95% CI -0.16 to -0.04 ml, p=0.001). Also, awakening cortisol levels after dexamethasone was associated with smaller hippocampal volume after adjusting for age and sex (B= -0.08 ml; 95% CI -0.14 to -0.02 ml, p=0.02). These findings did not materially change after adjustment for vascular risk and disease history. The association between higher evening cortisol and hippocampal volumes remained significant after additional adjustment for BPF (B= -0.089 ml; 95% CI -0.15 to -0.03 ml, p=0.002), and also for awakening cortisol after dexamethasone and smaller hippocampal volumes (B= -0.07 ml; 95% CI -0.13 to -0.01 ml, p=0.02) (Table 4). Repeating these analyses after excluding patients with major depression disorder (n=40) or dementia (n=4) showed that relations remained statistically significant (data not shown). When ICV was entered as covariate in the regression model with crude hippocampal volume, instead of the ratio hippocampus/ICV, the regression coefficients did not materially change and described relations remained significant (data not shown).

Table 4. Results of linear regression analysis of the associations of cortisol levels with relative hippocampal volumes.

	Hippocampal volume		
	B	**(95% confidence interval)**	**P value**
Awakening response			
Rise (SD = 7.8)			
Model I	-0.040	(-0.101 to 0.021)	0.20
Model II	-0.039	(-0.100 to 0.023)	0.22
Model III	-0.039	(-0.100 to 0.021)	0.21
AUC (SD = 6.9)			
Model I	-0.032	(-0.094 to 0.030)	0.31
Model II	-0.035	(-0.099 to 0.028)	0.28
Model III	-0.036	(-0.098 to 0.027)	0.26
Evening cortisol (SD = 4.2)			
Model I	-0.098	(-0.156 to -0.039)	0.001
Model II	-0.094	(-0.153 to -0.035)	0.002
Model III	-0.089	(-0.147 to -0.032)	0.002
Cortisol after dexamethasone (SD = 2.5)			
Model I	-0.075	(-0.138 to -0.016)	0.015
Model II	-0.071	(-0.133 to -0.010)	0.023
Model III	-0.070	(-0.130 to -0.010)	0.023

The coefficient B represents the difference (95% confidence interval) in hippocampal volume relative to ICV (ml) per standard deviation increase of rise, AUC, evening cortisol, and cortisol after dexamethasone, respectively.
Model I: Adjusted for age and sex
Model II: Model I additionally adjusted for smoking, alcohol intake, BMI, blood pressure, hyperlipidemia and diabetes mellitus, and disease history
Model III: Model II additionally adjusted for global brain atrophy

4. Discussion

We observed that higher cortisol levels in the evening and higher cortisol levels at awakening after dexamethasone were associated with smaller hippocampal volumes, independent of vascular risk factors and total brain volume in a large sample of middle-aged and elderly persons with a history of arterial disease. We found no association between the cortisol awakening response and hippocampal volume.

A strength of our study is the collection of multiple saliva samples that made it possible to examine different aspects of HPA-axis activity. Also, by giving clear instructions and having the patient record sampling times we maximized compliance, enabling us to report cortisol measures with respect to awakening time. The large number of subjects included, and the measurements of hippocampal and other brain volumes made it possible to obtain precise estimates. Also, we had information on important cardiovascular risk factors for which we could adjust in the analyses.

To our knowledge, this is the first study investigating the association of HPA-axis activity with hippocampal volume in such a large sample of subjects by assessing different aspects of the diurnal cortisol profile. Previous studies in humans found inconsistent results. Most of these studies were done in small sample sizes and used different methods to evaluate the basal HPA-axis activity. Studies finding smaller hippocampal volumes with higher levels of cortisol used 24-hour cortisol measurements in plasma (3) or urine, [30,39] whereas other studies using cortisol measurements in serum or saliva at predefined times as a marker of HPA-axis activity did not find an association. [21-23, 40] Since the evaluation of basal HPA-axis activity is complicated by its diurnal pattern these findings are difficult to interpret. When using a 24-hour measurement it is not possible to evaluate the different aspects of the diurnal profile. Sampling at predefined times, for example at 10AM, is subject to measurement variability because the cortisol secretion during the day and especially in the morning is related to the time of awakening. [33]

We found that higher levels in the evening and cortisol levels at awakening after the dexamethasone suppression, but not in the morning, were associated with smaller hippocampal volumes, independent of total brain volume. Higher levels of cortisol in the evening are generally linked to reduced feedback inhibition of the HPA-axis activity. [41] Two different corticosteroid receptors, the mineralocorticoid receptor and glucocorticoid receptor are involved in the feedback of cortisol. The mineralocorticoid receptor has a high affinity for cortisol and is thought to be involved in the regulation of the evening nadir of the diurnal rhythm, while glucocorticoid receptors are activated with increased levels of cortisol, for example during the morning peak or during acute stress. [42] Recent human data demonstrates that treatment with an mineralocorticoid receptor agonist results in a decrease in cortisol levels in the evening, while blockade of the mineralocorticoid receptor results in an increase of cortisol levels in the evening nadir, but not during the morning peak. [43-46] In the

primate brain, high levels of mineralocorticoid receptors are found in the hippocampal formation, while the glucocorticoid receptors are more ubiquitously distributed in the brain. [47] An explanation for our results of smaller hippocampal volumes with higher evening levels, but not morning levels may thus be that a decrease in hippocampal volume resulted in a decrease of density in mineralocorticoid receptors and therefore an increase in evening levels. This relationship was independent of total brain volume, which is suggestive of a specific association with the hippocampal volume. Since we do not have direct evidence for a positive relationship between hippocampal volume and receptor numbers this is a tentative explanation.

In contrast to recent studies suggesting a central role of the hippocampus in the morning response,[48-50] we did not find an association between the awakening response and hippocampal volume. These studies may not be comparable to our study sample. One study observed that bilateral hippocampal damage was associated with a lower awakening response. [50] Similar results were described in 18 patients with diabetes, in whom smaller volumes were associated with a blunted awakening response (49). A recent study reported the same association in healthy young men (n=13). [48] Also, using small sample sizes makes change finding more likely.

We found an association between higher awakening cortisol levels after suppression with dexamethasone and smaller hippocampal volumes. It has been suggested that reduced suppression of glucocorticoids after dexamethasone[21, 51-53] is associated with atrophy of brain regions rich in corticosteroid receptors, such as the hippocampus, the pituitary and hypothalamus. Animal studies showed that the synthetic glucocorticoid dexamethasone blocks HPA-axis activity primarily at the site of the pituitary instead of the hippocampus. [54,55] Although other studies failed to find an association between the dexamethasone suppression and hippocampal volumes, [14,21] our results suggest a role of the hippocampus in the negative feedback mechanism of the HPA-axis.

A limitation of our study is the cross-sectional design, which made it not possible to discern cause from consequence. Hippocampal atrophy has been described in various neuropsychiatric disorders that are associated with HPA-axis dysregulation, such as depression, [16] PTSD[56] and Alzheimer's disease. [57] It has frequently been hypothesized that HPA-axis dysregulation plays a causal role in hippocampal volume reduction. [5] The neurotoxicity of cortisol acts via corticosteroid receptors [58] and this anatomical specificity may be due to high concentration of corticosteroid receptors in the hippocampus. [47] Therefore, prolonged high levels of cortisol in the evening may have adverse effects on the hippocampal volume. And, since the hippocampus also plays a inhibiting role in the HPA-axis activity, this damage may result in diminished inhibition of glucocorticoids. [7]. A recent prospective study reported that higher levels in the evening predicted decline in hippocampal dependent memory function, indicating that high levels of cortisol in the evening may precede hippocampal damage. [59] However, our results may also be suggestive of higher evening levels being

a consequence of hippocampal volume loss if a decrease in hippocampal volume resulted in a decrease of density in MRs and therefore an increase in evening levels. One study suggestive of this reversed causality reported alterations in morning cortisol rhythm compared to control subjects in patients with overt hippocampal damage due to encephalitis or surgery. [50] An alternative explanation might be that both hippocampal volume and cortisol levels are independently associated with a third variable. For example, cytokines have been associated with both neurodegeneration and HPA-axis activation. [60-62]

We tried to maximize compliance by having the patient record the sampling time. Still, we cannot exclude the possibility of non-adherence to the sampling protocol in some persons. However, these limitations concerning the reliability of the sampling are expected to be compensated by the large sample size. Furthermore, there may have been selective non-compliance of saliva sampling. Perhaps subjects with smaller hippocampal volumes had more memory problems and were less compliant with the saliva sampling protocol. However, the mean hippocampal volume in the excluded subjects did not differ from the study sample. Another limitation is that we did not measure serum dexamethasone levels. There may be variability in serum dexamethasone levels after ingesting dexamethasone and dexamethasone metabolism may be a determinant of cortisol levels. [63,64]

Although the observed differences in hippocampal volumes seem small, they are comparable to differences in evening cortisol levels found between healthy controls and patients with depression, [65] PTSD and dementia. [66,67]

Our study population consisted of patients with a history of arterial disease. Since several vascular risk factors have been associated with altered basal HPA-axis activity and brain atrophy, [68] we cannot exclude the possibility that the observed associations are restricted to patients with arterial disease. However, examining the relation between basal HPA-axis activity and hippocampal volumes in a population with high vascular burden may have made it more likely to find an association that may not have been observed in younger and healthier populations.

In conclusion, we observed that higher cortisol levels in the evening and higher cortisol levels after dexamethasone suppression at awakening were associated with smaller hippocampal volumes, independent of total brain volume. Prospective studies should determine to what extent elevated cortisol secretion in the evening and reduced suppressibility of the HPA-axis is a consequence of smaller hippocampal volume or whether it increases the risk for hippocampal volume loss and development of Alzheimer's disease.

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4.2 Hippocampal and entorhinal cortex volumes and role of hypothalamic pituitary adrenal axis in depression. The SMART-Medea study

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Abstract

Context

Structural brain changes have often been found in major depressive disorder (MDD) and it is thought that hypothalamic-pituitary-adrenal (HPA) axis hyperactivity may explain this relation.

Objective

To investigate the association of MDD and history of depressive episodes with hippocampal and entorhinal cortex volumes, and whether HPA-axis activity explains this association.

Design Cross-sectional observational study.

Participants

636 participants with a history of atherosclerotic disease (mean age 62±9 years, 81% male) from the Second Manifestation of ARTerial disease-Memory depression and aging (SMART-Medea) study. Twelve-month diagnosis of MDD, and history of depression was assessed using the Composite International Depression Interview. Age of first depressive episode was classified into early-onset depression (EOD) (<50 years) and late-onset depression (LOD) (≥50 years). HPA-axis activity was assessed by salivary cortisol. The cortisol awakening response was defined as the area under the curve; resting levels as the average of the samples taken at 10PM and 11PM. Suppression of the HPA-axis was defined as the cortisol value at awakening after ingestion of 0.5 mg dexamethasone at 11PM.

Main outcome measures

Manually outlined volumes of hippocampus and entorhinal cortex on 1.5 Tesla magnetic resonance images relative to intracranial volume.

Results

General linear models adjusted for demographics, vascular risk, antidepressant use and white matter lesions showed that MDD was not associated with hippocampal or entorhinal cortex volumes. Participants with EOD had smaller hippocampal volume than those never depressed (adjusted mean difference -0.06 ml; 95% CI -0.001 to -0.123 ml), whereas participants with LOD had smaller entorhinal cortex volume (adjusted mean difference -0.009 ml; 95% CI -0.001 to -0.017 ml). HPA-axis activity did not explain these differences.

Conclusion

We found differential associations of age of onset of depression on hippocampal and entorhinal cortex volumes, which could not be explained by HPA-axis hyperactivity.

1. Introduction

Structural brain abnormalities have often been found in major depressive disorder (MDD) and it is thought that these are involved in the underlying mechanisms of MDD.[1;2] Particularly the hippocampus has received a lot of interest, as the hippocampus is part of the limbic system and plays a role in memory function and emotion regulation.[3;4] Several studies, although not all, observed smaller hippocampal volumes in patients with MDD compared with healthy controls.(for review see:[5-7]) Recent studies suggest that particularly severe and recurrent depression are associated with hippocampal atrophy.[1;6]

It is also suggested that the age of onset of first depressive episode is of importance in the association between depression and structural brain abnormalities [8-10]. An early onset of depression (EOD) is associated with recurrent depression, and may therefore be associated with hippocampal atrophy, whereas a late onset of depression (LOD) more likely is associated with vascular brain pathology, like white matter hyperintensities.[11-13] Thus far, inconsistent findings have been reported, as smaller hippocampal volumes have been found in EOD[14;15], but also in LOD.[16;17]

An often proposed explanation for the relation between MDD and smaller hippocampal volumes is hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis that may occur in MDD.[18;19] The hippocampus plays an inhibitive role in regulating the HPA-axis[20] and chronic exposure to glucocorticoids with repeated depressive episodes could lead to cell death and hippocampal atrophy.[21] Evidence for glucocorticoid-mediated hippocampal volume loss is scarce in human studies. Recently, we reported that higher evening levels of cortisol and higher levels at awakening after ingesting 0.5 mg dexamethosone were associated with smaller hippocampal volumes.(Knoops et al, in revision Biol Psychiatry), although others found no relation between cortisol measures and hippocampal volume.[22;23] Moreover, one study among elderly depressed patients did not find that cortisol levels mediated the association between depression and smaller hippocampal volumes.[13]

The entorhinal cortex is also part of the medial temporal lobe and also regulates memory function.[24;25] It has been proposed that volumetric abnormalities in the entorhinal cortex lead to impairments of the cortical-hippocampal circuit and these structural changes have been implicated in the aetiology of depression.[26] Although the entorhinal cortex and hippocampus are closely related, the entorhinal cortex has hardly been investigated in relation to depression. One recent study reported that in women, treatment resistant depression was associated with smaller entorhinal cortex volumes,[27] but still much is unknown of the relation between depression and entorhinal cortex volume and it is also unclear to which extent the entorhinal cortex is associated with HPA-axis activity.

To our knowledge, no previous studies examined MDD and history of depressive episodes with hippocampal and entorhinal cortex volumes in a single

population. Also, only a few studies examined the role of HPA-axis activity in this relationship. The aim of this study was threefold. First, we investigated whether MDD, severity of depressive symptoms, and age of onset of depressive episodes were associated with hippocampal and entorhinal cortex volumes. Secondly, we examined the separate associations of depression measures with HPA-axis activity, and HPA-axis activity with entorhinal cortex volumes. Thirdly, we investigated to what extent HPA-axis activity explained or mediated the association of depression with hippocampal and entorhinal cortex volumes. We did so in a large cohort of participants with a history of atherosclerotic disease, because these participants are at increased risk for depression,[28;29] HPA-axis dysregulation,[30;31] and brain atrophy[32;33] and may thus be more vulnerable for the potential detrimental effects of depression on hippocampal and entorhinal cortex volumes.

2. Methods

Subjects

Data were used from the Second Manifestations of ARTerial disease-Magnetic Resonance (SMART-MR) study, a prospective cohort study aimed to investigate brain changes on MRI in 1309 independently living participants with symptomatic atherosclerotic disease. Details of the design and participants have been described elsewhere.[34-36] In brief, between May 2001 and December 2005, all patients newly referred to the University Medical Center Utrecht with manifest coronary artery disease, cerebrovascular disease, peripheral arterial disease or an abdominal aortic aneurysm (AAA), and without MR contraindications were invited to participate.

Between January 2006 and May 2009, all participants still alive were invited for follow-up measurements, including MRI of the brain, neuropsychological testing, a physical examination, blood and urine sampling, risk factors, medical history, and functioning. In addition, as part of the SMART-Medea (Memory, depression and aging) study, an ancillary study to the SMART-MR study, aimed to investigate brain changes associated with psychosocial vulnerability and stress factors, measurements of salivary cortisol and psychosocial stressors early and later in life were added. From March 2006, diagnostic assessment of depression and a T1-weighted 3-dimensional fast field-echo sequence for measuring hippocampal and entorhinal cortex volumes were added. The SMART-MR and SMART-Medea study were approved by the ethics committee of our institution and written informed consent was obtained from all participants.

In total, 754 of the surviving cohort (61% of n=1,238) gave written informed consent and participated at follow-up; 466 (38%) persons refused did not respond and 18 (1%) were lost to follow-up.

Depression measures

The presence of major depressive disorder (MDD) in the preceding 12 months was assessed in all participants according to DSM-IV criteria[37] using the Composite International Depression Interview (CIDI, version 2.1).[38]

History of depression was based on affirmative answers to one of the two core symptoms of the CIDI lifetime depression section, and the age of first depressive episode was assessed. Participants who had their first episode prior to the age of 50 years were classified as having early-onset depression (EOD), whereas participants who had experienced their first episode at age 50 years or older were classified as having late-onset depression (LOD). This cut-off was chosen, because in a previous study this cut-off was proven to be appropriate in detecting differences between age of onset groups.[16] We created three depression groups: 12-month MDD; remitted (participants with a history of depressive episodes, but no 12-month diagnosis), and never depressed. In subsequent analyses, we differentiated the depressed and remitted persons in those with EOD and LOD to examine the role of age of onset.

Severity of symptoms in the past two weeks was measured with the Patient Health Questionnaire (PHQ-9),[39;40]which assesses the presence of the nine DSM-IV criteria for MDD on a 4-point scale, ranging from 0 ("not at all") to 3 ("nearly every day") (total score range 0 to 27).

Magnetic Resonance Imaging Protocol

The MR investigations were performed on a 1.5-Tesla whole-body system (Gyroscan ACS-NT, Philips Medical Systems, Best, the Netherlands). The protocol consisted of a transversal T1-weighted gradient-echo sequence (repetition time (TR)/echo time (TE): 235/2 ms; flip angle, 80º), a transversal T2-weighted turbo spin-echo sequence (TR/TE: 2200/11 ms and 2200/100 ms; turbo factor 12), a transversal T2-weighted fluid attenuating inverse recovery (FLAIR) sequence (TR/TE/inversion time (TI): 6000/100/2000 ms) and a transversal inversion recovery (IR) sequence (TR/TE/TI: 2900/22/410 ms) (field of view 230x230 mm; matrix size, 180x256; slice thickness, 4.0 mm; no gap; 38 slices). Furthermore, for measurements of the hippocampal and entorhinal cortex volumes, we obtained a sagittal T1-weighted 3D FFE (fast field echo) sequence (TR/TE: 7.0/3.2 ms; flip angle, 8º), FOV 240 mm; matrix size, 240×256; slice thickness, 1.0 mm; no gap; 170 slices).

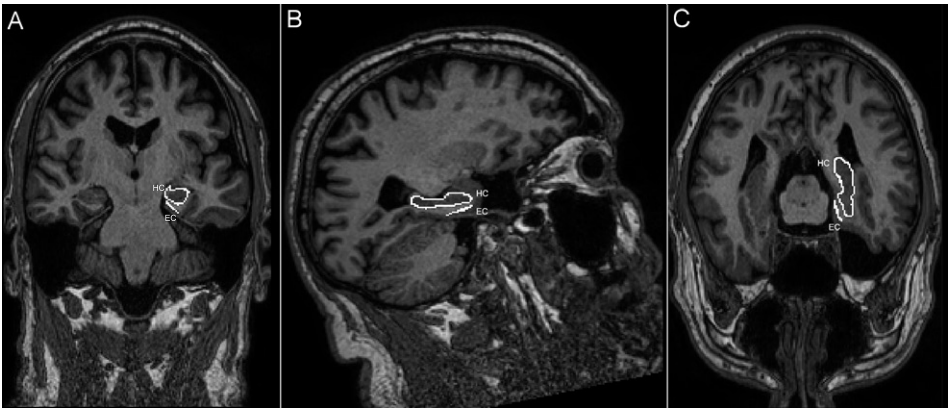
Assessment of hippocampal and entorhinal cortex volume

The sagittal T1-weighted images were tilted to the coronal plane and orientated perpendicular to the long axis of the left hippocampus. Measurements of hippocampal volumes were performed by two trained investigators (AJGK and LG), blinded to all clinical information. The hippocampus was manually outlined on an average of 40 slices and included the hippocampus proper, subiculum, fimbria, alveus, and dentate gyrus. We started at the first slice where the hippocampus was visible as the anterior

boundary. The alveus, which was clearly visible, formed the dorsal border of the hippocampus on the anterior slices and was used to separate the hippocampal head from the amygdala. More posterior, the dorsal boundary was defined by CSF and choroid plexus, which was not included in the measurements. The posterior border was defined as the slice before the total length of the fornix was visible. The lateral boundaries were defined by CSF of the temporal horn of the lateral ventricle and by the gray-white matter border of the temporal stem. Medially, the hippocampus was bounded by CSF in the cisterna ambiens and transverse fissure, and ventrally by white matter of the parahippocampal gyrus (Figure 1).

All image processing for the entorhinal cortex was performed by one investigator (LG) blinded to all clinical information. The entorhinal cortex was manually outlined on an average of 19 slices. For determining boundaries of the entorhinal cortex we used criteria that were based on the cortical topographic landmarks on MRI that best approximated histologically determined cytoarchitectural boundaries of the entorhinal cortex.[41] We applied some minor modifications in order to improve reliability, as previously described by Goncharova and colleagues.[42] In short, the rostral end of the hippocampus was used to define the rostral extent of the entorhinal cortex rather than the sulcus semi-annularis, because the sulcus semi-annularis was not always clearly depicted on MRI. The posterior boundary of the entorhinal cortex was the first image section containing the intralimbic gyrus when progressing from posterior to anterior. Its inferolateral boundary was defined by the medial edge of the collateral sulcus posteriorly and the rhinal sulcus anteriorly. In some cases, this sulcus was bi- or multi-lobed. In these sections, the more medially located sulcus was used as the border. The medial boundary was CSF in the crural cistern. The entorhinal cortex was differentiated from the subiculum by a medial extension of the horizontal line defined by the gray–white matter subicular interface (Figure 1).

Figure 1. Magnetic resonance images of the hippocampal formation and entorhinal cortex



Shown are coronal (panel A), sagittal (panel B) and axial (panel C) images.
HC = Hippocampus; EC = entorhinal cortex.

Left and right entorhinal cortex and hippocampal volumes were calculated by multiplying the total number of voxels by the volume of a voxel (1.0x0.94x0.94 mm). The intra-rater reliability coefficient for repeated tracing in 20 randomly selected hippocampi was 0.96 and 0.98, and the inter-rater agreement between the two raters was 0.96. The intra-rater reliability coefficient for repeated tracing in 20 randomly selected entorhinal cortex was 0.92. The coefficient of variation[43] for the two raters was 3.8%. The mean difference in hippocampus volume measurements between the two raters was 11.1 voxels. For the entorhinal cortex the mean difference in volume between the two measurements was 18 voxels and the coefficient of variation was 4.8%.

HPA-axis activity

HPA-axis activity was assessed at home by 7 measurements of cortisol in saliva over a period of 24 hours to obtain the circadian rhythm.[44] The saliva was collected using cotton dental rolls (Salivette, Startstedt). Participants were instructed to refrain from smoking, drinking caffeine, eating or cleaning their teeth at least 30 minutes before collecting saliva, and to chew on the rolls for at least 2 minutes. On day 1, participants were instructed to take the first sample immediately after awakening while still lying in bed, and to take the second, third and fourth samples after 30, 45 and 60 minutes. Sample 5 and 6 were collected at 10PM and 11PM, respectively. Furthermore, participants were asked to take 0.5 mg of dexamethasone orally after their sixth saliva sample, and to sample their saliva the next morning directly after awakening. Participants were asked to record the time at which each saliva sample was taken. They were instructed to sample on a regular weekday and to store their saliva in their freezers until the day of the visit. At the lab, the saliva samples were centrifuged at 3000 rpm for 10 minutes and then stored at -20^{o}C until assayed. The cortisol in saliva was measured without extraction using an in house competitive radio-immunoassay employing a polyclonal anticortisol-antibody (K7348). [1,2-^{3}H(N)]-Hydrocortisone (NET185, NEN - DUPONT, Dreiech, Germany) was used as a tracer. The lower limit of detection was 0.5 nmol/L and inter-assay variation was 9% at 3 nmol/L and 5% at 23 nmol/L. Intra-assay variation was 4%.

The cortisol awakening response was assessed by calculating the area under the curve to the ground (AUCg).[45] We also calculated the area under the curve with respect to increase (AUCi), but since this measure did not result in different findings and was highly correlated to AUCg (r=0.55), we only used the AUCg. Second, resting levels of cortisol were defined as the average of the saliva samples taken at 10PM and 11PM. Third, as an indicator of suppression of the HPA-axis, the cortisol value was taken at awakening the morning after the ingestion of the dexamethasone. Because evening cortisol levels and awakening cortisol after the dexamethasone suppression test (DST) were skewed these data were natural log-transformed.

Other variables

Total brain volume, including the cerebrum, brainstem and cerebellum, was calculated by summing the volumes of grey and white matter and, if present, white matter lesions (WML) and infarcts.[34;46] Total intracranial volume (ICV) was calculated by summing total brain volume and volumes of sulcal and ventricular cerebrospinal fluid. Educational level was divided into eight categories, graded from primary school to academic degree, according to the Dutch educational system. During the patient's visit to the medical center, an overnight fasting venous blood sample was taken to determine glucose levels. Height and weight were measured without shoes and heavy clothing, and the body mass index (BMI) was calculated (kg/m^2). Systolic and diastolic blood pressures (mm Hg) were measured three times with a sphygmomanometer and averaged. Diabetes mellitus was defined as a history of diabetes mellitus, glucose ≥7.0 mmol/L or self reported use of oral antidiabetic drugs or insulin. Smoking habits, alcohol intake, and antidepressant use (yes vs. no) were assessed with questionnaires. Pack years of smoking was calculated and alcohol use was categorized into <1 drink p/ week, 1-20 drinks p/week, and >20 drinks p/week.

Study sample

Of the 754 participants who were examined between 2006 and 2009, a 3D T1-weighted MRI of the brain was made in 649 participants and 636 scans were without artefacts. The hippocampus was manually outlined on these 636 scans, and the entorhinal cortex was outlined in all participants with MDD (N=47) and a random subset of all other scans (N=432). The sample in which entorhinal cortex volume was not outlined (N=157) did not differ on any of the covariates from the sample in which the entorhinal cortex was outlined (N=479) ($p > 0.05$).

Data-analysis

Missing data rarely occur at random and a complete case analysis (deletion of all participants with one or more missing values) leads to loss of statistical power and to biased results. We therefore used multiple imputation (10 datasets) to address the missing values[47-49] using the statistical programme R (version 2.10.0). Data were analyzed using SPSS version 17.0 (Chicago, Ill, USA), by pooling the 10 imputated datasets.

Left and right hippocampal and entorhinal cortex volumes and the average of left and right were analyzed. Volumes were divided by ICV[50] and multiplied with the average ICV of the study population (1455 ml) to obtain relative volumes. In separate analyses, volumes were also expressed relative to total brain volume to examine whether relationships with hippocampal or entorhinal cortex volumes were independent of global brain atrophy.

Depression measures with hippocampal and entorhinal cortex volumes

First, general linear models were created to estimate adjusted mean differences in hippocampal and entorhinal cortex volumes according to the three depression groups (MDD, remitted, never depressed). Second, we estimated the adjusted mean differences in hippocampal and entorhinal cortex volumes according to history of depression (EOD vs. no history; LOD vs. no history). Third, multiple linear regression analysis was used to estimate the association of depressive symptoms as measured with the PHQ-9 with hippocampal and entorhinal cortex volumes. All analyses were adjusted for age, sex, education, BMI, diabetes mellitus, systolic and diastolic blood pressure, smoking habits, alcohol intake, antidepressant use and WML volumes (relative to ICV).

Role of HPA-axis

Using general linear models we estimated adjusted mean differences in cortisol levels according to the three depression groups. We also estimated the adjusted association of tertiles of cortisol levels with entorhinal cortex volume as dependent variable. Analyses were adjusted for age, sex, education, BMI, diabetes mellitus, systolic and diastolic blood pressure, smoking habits, alcohol intake, antidepressant use, and time of awakening. To investigate whether HPA-axis activity explained or mediated a relation between depression and medial temporal lobe volumes, cortisol measures were added to the models with depression measures as independent variables and hippocampal and entorhinal cortex volumes as dependent variables. Also, interactions between cortisol levels and depression measures were tested. These analyses were adjusted for age, sex, education, BMI, diabetes mellitus, systolic and diastolic blood pressure, smoking habits, alcohol intake, antidepressant use and WML volumes (relative to ICV).

3. Results

Table 1 presents the characteristics of the study population according to depression status. MDD was diagnosed in 7.4% of the population; 37.6% reported a history of depressive episodes but were in remission; and 55% were never depressed.

Table 1. Baseline characteristics according to depression group

	Never N=350	Remitted N=239	MDD N=47	Missing values %
Demographics				
Age	62 (9)	62 (9)	58 (10)	0
Male %	82	81	76	0
Level of education (0-8)	4 (2)	4 (2)	4 (2)	1
Vascular risk factors				
Body mass index	28 (4)	27 (4)	28 (4)	< 1
Systolic blood pressure (mmHg)	144 (19)	142 (19)	143 (20)	< 1
Diastolic blood pressure (mmHg)	82 (11)	82 (11)	84 (11)	< 1
Diabetes Mellitus %	23	19	16	2
Smoking (packyears)[a]	16 (0 – 38)	23 (0 – 52)	21 (0 – 49)	3
Alcohol use %				0
< 1 drinks p/week	31	30	36	
1 – 20 drinks p/week	58	59	55	
> 20 drinks p/week	11	11	9	
Brain measures				
Intracranial volume (ml)	1454 (126)	1465 (106)	1442 (147)	0
Total brain volume (ml)	1139 (105)	1145 (106)	1137 (119)	0
White matter lesion volume (ml)[a]	1.3 (0.3 – 9.6)	1.3 (0.3 - 6.5)	1.4 (0.4 – 7.0)	0
Crude hippocampal volume (ml)				
Left	2.97 (0.36)	2.94 (0.38)	2.90 (0.32)	0
Right	3.00 (0.39)	3.00 (0.43)	2.91 (0.35)	0
Crude entorhinal cortex volume (ml)[b]				
Left	0.17 (0.03)	0.17 (0.03)	0.17 (0.03)	0
Right	0.17 (0.03)	0.17 (0.03)	0.17 (0.03)	0
Depression characteristics				
Depressive symptoms[a,c]	1.0 (0.0 – 5.0)	2.0 (0.0 – 8.0)	7.0 (2.2 – 17.0)	2
Age of onset of depression	NA	40 (14)	43 (17)	2
Use of antidepressants %	4	8	31	0
Early onset of depression %	NA	74	64	0
Late onset of depression %	NA	26	36	0
HPA-axis activity				
AUCg morning cortisol nmol/L*hour	17.4 (7)	17.7 (8)	17.0 (7)	17
Mean evening cortisol nmol/L[a]	3.4 (2.0 – 6.2)	3.1 (1.8 – 7.9)	3.8 (2.3 – 7.5)	12
Awakening cortisol after DST nmol/L[a]	1.6 (0.9 – 3.5)	1.9 (0.7 – 3.1)	1.8 (0.6 – 3.0)	19

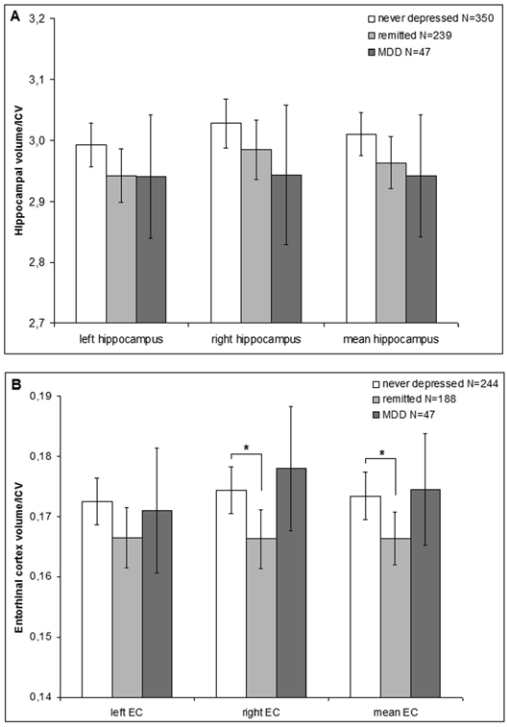
Data presented as means with standard deviations unless otherwise specified.
a: data presented as medians with 10-90% intervals; b: assessed in 479 participants; c: measured with the Patient Health Questionaire-9.
MDD=major depressive disorder; HPA= Hypothalamic-Pituitary-Adrenal; AUCg = Area Under the Curve to the ground; DST=Dexamethasone Suppression Test

Depression measures with hippocampal and entorhinal cortex volumes

Participants with a 12-month diagnosis of MDD did not have significantly smaller hippocampal volumes (adjusted mean difference -0.07 ml; 95% CI -0.18 to 0.04 ml; p=0.21) or entorhinal cortex volumes (adjusted mean difference -0.001 ml; 95% CI -0.011 to 0.009 ml; p=0.84) than those who were never depressed (Figure 2). Those with remitted depression had statistically significantly smaller entorhinal cortex volumes than those who were never depressed (adjusted mean difference -0.008 ml; 95% CI -0.015 to -0.001 ml; p=0.02), but no significant difference was found on hippocampal volumes (adjusted mean difference -0.05; 95% CI -0.10 to 0.01 ml; p =0.10).

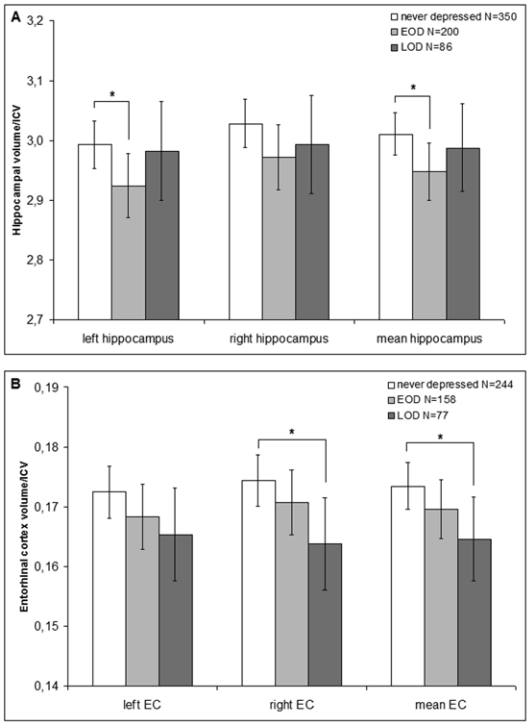
We further explored the role of age of onset of depression by differentiating EOD and LOD within participants who were remitted or had MDD. The observed smaller entorhinal cortex volumes reached significance in the LOD group. The adjusted mean difference in average entorhinal cortex volume between LOD and never depression was -0.009 ml (95% CI -0.017 to -0.001 ml; p=0.03), whereas no significant differences in entorhinal cortex volumes were found for EOD vs. never depression (Figure 3). In contrast, subjects with EOD had significantly smaller hippocampal volumes than those who were never depressed (adjusted mean difference in average hippocampal volume -0.06 ml; 95% CI -0.123 to -0.001ml; p=0.04).

Figure 2. Adjusted means of hippocampal (A) and EC (B) volumes, divided by ICV according to depression groups



Error bars represent 95% confidence intervals; *p < 0.05
Mean volumes (in ml) are adjusted for age, sex, level of education, blood pressure, diabetes mellitus , body mass index, pack years of smoking, alcohol consumption, antidepressant use and volume of white matter lesions. EC= entorhinal cortex ICV= intracranial volume

Figure 3. Adjusted means of hippocampal (A) and EC (B) volumes, divided by ICV, according to age of onset of first depressive episode.



Error bars represent 95 % confidence intervals; *p < 0.05
Mean volumes (in ml) are adjusted for age, sex, level of education, blood pressure, diabetes mellitus, body mass index, pack years of smoking, alcohol consumption, antidepressant use and volume of white matter lesions.
EC=entorhinal cortex ICV=intracranial volume EOD= early-onset depression LOD=late-onset depression

Compared to associations found with volumes divided by ICV, the associations of volumes divided by total brain volume were slightly weaker, but the relation between LOD and entorhinal cortex remained significant (adjusted mean difference -0.009 ml; 95% CI -0.017 to 0.00 ml; p=0.04), whereas the relation between EOD and mean hippocampal volume was no longer significant (adjusted mean difference -0.05 ml; 95% CI -0.11 to 0.01 ml; p=0.10). To appreciate the magnitude of the volume differences Table 2 shows the percentual differences per depression group. As can be seen, the percentual differences on hippocampal volume ranged from -2.8 % with MDD to -0.3 % with LOD, while the percentual differences on entorhinal cortex volume ranged from -6.1 % with LOD to +2.1 % with MDD.

Fully adjusted linear regression models showed that depressive symptoms were not associated with hippocampal volumes (left: B per point increase on PHQ-9 -0.001 ml; 95% CI -0.01 to 0.01; p=0.89 and right: B 0.003 ml; 95% CI -0.01 to 0.01; p=0.53), or with entorhinal cortex volumes (left: B -0.001 ml; 95% CI -0.001 to 0.000; p=0.21 and right: B 0.000 ml; 95% CI -0.001 to 0.001; p=0.45).

Table 2. Percentual differences in hippocampal and EC volumes per depression group.

	12-month MDD	Remitted	EOD	LOD
Hippocampal volume				
- Mean	-2.3 %	-1.6 %	-2.1 %*	-0.8 %
- Right	-2.8 %	-1.4 %	-1.9 %	-1.2 %
- Left	-1.7 %	-1.7 %	-2.3 % *	-0.3 %
Entorhinal cortex volume				
- Mean	+0.6 %	-4.0 %*	-2.3 %	-5.1 %*
- Right	+2.1 %	-4.6 % *	-2.1 %	-6.1 % *
- Left	-0.9 %	-3.5 %	-2.4 %	-4.2 %

* $p < 0.05$

MDD= major depressive disorder; EC= entorhinal cortex; EOD= early-onset depression; LOD= late-onset depression

Role of HPA-axis

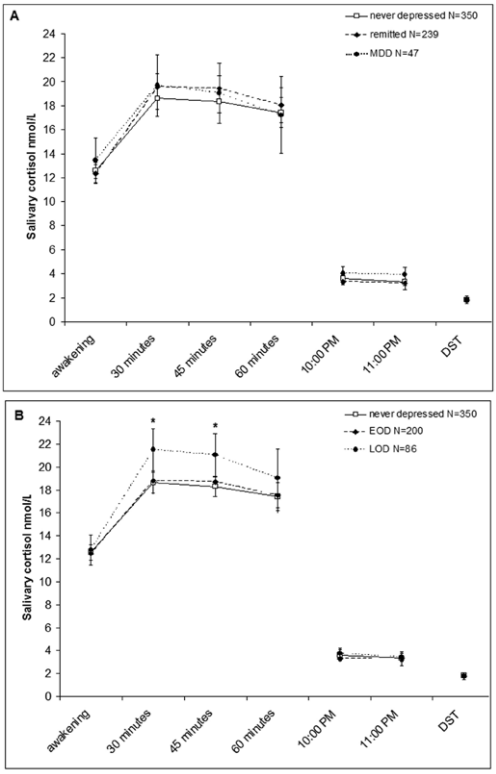
The three depression groups did not differ in levels of cortisol (Figure 4A). However, compared to subjects who were never depressed, LOD was associated with higher AUCg (adjusted mean difference 2.01 nmol/L*hour; 95% CI 0.42 to 3.60; p=0.013), while EOD was not (p=0.80) (Figure 4B). LOD and EOD were not associated with evening cortisol levels or awakening cortisol after DST (p >0.05).

Recently, we showed that high evening cortisol and high awakening cortisol after DST were associated with smaller hippocampal volumes.(Knoops et al, in revision Biol Psychiatry) In addition to this, we found that higher evening cortisol levels were also associated with smaller right entorhinal cortex volume (B for highest vs. lowest tertile of evening cortisol -0.011 ml; 95% CI -0.019 to -0.003 ml; p=0.008), but not with

left entorhinal cortex volume (B = -0.001 ml; 95 % CI -0.01 to 0.01; p=0.80) or with mean entorhinal cortex volumes (B= -0.006; 95 % CI =-.01 to 0.001; p=0.10). Morning cortisol (AUCg) and awakening cortisol after DST were not significantly associated with entorhinal cortex volumes (results for mean entorhinal cortex volumes were B for highest vs. lowest tertile of AUCg morning cortisol 0.005; 95 % CI 0.003 to 0.01; p=0.21 and B for highest vs. lowest tertile of awakening cortisol after DST -0.006 ml; 95% CI -0.0194 to 0.001 ml; p=0.78).

When cortisol measures were added to the regression models to investigate whether HPA-axis activity explained the relation of depression measures with brain volumes, none of the associations altered. Also, we found no evidence for cortisol-mediated hippocampal or entorhinal cortex volume loss in depression as none of the interaction terms between depression and cortisol measures reached significance (p-value for interaction terms >0.05).

Figure 4. Adjusted salivary cortisol levels according to depression groups (A) and age of onset of depression (B).



Error bars represent 95 % confidence intervals; * p < 0.05
Mean levels are adjusted for age, sex, educational level, systolic and diastolic blood pressure, diabetes mellitus, body mass index, pack years of smoking, alcohol consumption, antidepressant use and time of awakening.

4. Discussion

In this large cohort study among participants with a history of atherosclerotic disease MDD was not associated with smaller hippocampal or entorhinal cortex volumes. A differential association was observed for early- and late-onset depression; where LOD was associated with smaller entorhinal cortex volumes, and EOD with smaller hippocampal volumes. HPA-axis hyperactivity did not explain these associations.

To our knowledge this study is the largest cohort study investigating hippocampal and entorhinal cortex volumes in relation to DSM-IV diagnoses of MDD. Many previous studies, although not all, found that MDD was associated with smaller hippocampal volumes[5-7] and some also with smaller entorhinal cortex volumes.[27;51] Most of these studies had case-control designs and compared in- or outpatients with MDD with healthy controls. As a consequence the contrast between cases and controls was larger than in our study, making it easier to detect differences. However, case-control designs are highly prone to selection bias and the observed differences may be overestimated. Also, many studies did not adjust for possible confounders or did not examine volumes relative to intracranial or total brain volume.

To our knowledge, the relation between a history of depression and entorhinal cortex volumes has not been investigated before. We found a 5.1% reduction in entorhinal cortex volume in participants with LOD, whereas this difference was only 0.8% in hippocampal volume and in participants with EOD the reduction was 2.3% and 2.1% in entorhinal cortex and hippocampal volume, respectively.

It has been suggested that EOD and LOD differ in aetiology; EOD is thought to be a result of the interplay between genetic predisposition and stressful experiences, whereas LOD is thought to be a result of vascular brain pathology.[9;14] However, because we adjusted for vascular risk factors and white matter lesions the association between LOD and smaller entorhinal cortex volumes cannot be explained by underlying vascular brain pathology. Another explanation for our findings might be that the LOD in our study is a consequence of loss of entorhinal cortex volume. A differential association of hippocampal and entorhinal cortex volumes has been observed in relation to Alzheimer's disease, where it is thought that the entorhinal cortex is affected in an earlier stage of Alzheimer's disease pathology than the hippocampus.[50;52-54] Possibly, LOD represents a prodromal phase of Alzheimer's disease. [55-5] Our findings that EOD, but not LOD, was associated with smaller hippocampal volumes, are in line with results from a preceding study.[11] Other studies also failed to find a relation between LOD and hippocampal volume,[60;61] whereas other studies found more hippocampal volume loss in LOD than in EOD.[16;17] EOD is associated with recurrent depression and stress-related neurotoxic factors associated with repeated episodes of depression may have resulted in a smaller hippocampal volumes in our study.[62;63]An alternate possibility is that smaller hippocampal volumes antedates the onset of depression early in life, as has also been suggested in the onset of post-traumatic stress disorder. [64]This hypothesis

has hardly been investigated, although one study found that discordant twins in their risk for depression and anxiety differed in hippocampal volume, with high risk twins having smaller hippocampal volumes than the low risk twins. [65] Based on these findings it seems likely that hippocampal volume is affected by environmental factors and unlikely that smaller hippocampal volumes constitute a risk for onset of depression. However this study was fairly small, so further studies are needed to investigate whether hippocampal volume loss is a cause or a consequence of depression.

Both depression and Alzheimer's disease have frequently been associated with HPA-axis dysregulation[18;19;66;67] and HPA-axis hyperactivity may underlie the association between depression and hippocampal atrophy and risk for Alzheimer's disease.[21;56] However, we found no evidence for glucocorticoid mediated hippocampal and entorhinal cortex volume loss, which is in line with two preceding studies.[13;68] Although LOD was associated with higher awakening cortisol, awakening cortisol was not associated with entorhinal cortex or hippocampal volumes. Also, adding depression and cortisol measures to the same model did not change the associations.

Our study has several strong aspects one of which is that we were able to investigate whether HPA-axis hyperactivity explained the observed relations. The collection of multiple saliva samples made it possible to examine different aspects of HPA-axis activity. Also, by giving clear instructions and having the patient record the sampling time we maximized the compliance and this enabled us to report cortisol measures with respect to the awakening time. Furthermore, we included the age of onset of first depressive episodes and severity of depressive symptoms. Hippocampal and entorhinal cortex volumes were manually outlined on MRI images with isotropic voxels, making it possible to measure these structures in great detail. We had data on important covariates, for which associations could be adjusted and we had precise measurements of brain volumes, which made it possible to adjust associations for ICV, total brain volume and white matter lesions.

A limitation of our study is the cross-sectional design and therefore cause and consequence cannot be discerned. By assessing history of depression we tried to retrospectively investigate whether depression preceded brain volume loss, but the smaller brain volumes could still represent a vulnerability to the development of depression. Also, history of depression was assessed using only the two core questions of the CIDI and some participants will not have fulfilled DSM-IV criteria for history of MDD. This misclassification may have diluted the associations for history of depression.

Our study population consisted of patients with a history of atherosclerotic disease. We thus do not know if the observed associations can be generalized to the general population. However, examining the relation between depression, HPA-axis activity and brain volumes in a population with high vascular burden may have made it more likely to find an association that may not have been observed in younger and healthier populations.

To summarize, in this cohort MDD was not associated with hippocampal or entorhinal cortex volumes. LOD was associated with smaller entorhinal cortex volumes, whereas EOD was associated with smaller hippocampal volumes. We found no evidence for glucocorticoid-mediated hippocampal or entorhinal cortex volume loss in depression. Prospective studies are needed to further unravel the relation between depression, HPA-axis activity and brain volumes.

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Chapter 5

General Discussion

5

5. General Discussion

Stress and the hypothalamic-pituitary-adrenal axis

Already in 1936, the term stress was introduced in medical science, when the endocrinologist Hans Selye borrowed the term from engineering to refer to physical responses to a wide variety of deleterious agents.[1] Selye investigated various physical stressors (e.g. fasting, extreme cold, operative injuries and drug administration) and concluded that many conditions can put strain on the organism and lead to disease the same way that many unspecific conditions can put strain on a piece of metal and break it like glass. In addition to the research by Selye, John Mason examined stress hormones in humans during various stressful conditions (air-traffic controlling or parachute jumping).[2] He concluded that there are three main features which induce the stress response, namely 1) the event has to be novel, 2) unpredictable and/or 3) the individual must have the feeling that he/she does not have control over the situation

When an event is interpreted as being stressful, it triggers the activation of the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal (HPA) axis. The SNS is responsible for the rapid stress response, involving the release of catecholamines (adrenaline and noradrenaline) within seconds of the onset of the stressor, whereas, the HPA axis is responsible for the slower onset stress response, involving the release of glucocorticoids (cortisol in humans) by the adrenal cortex. Cortisol follows a specific circadian rhythm, with peak levels 30 min after awakening when cortisol levels increase by 50–60% [3] and a nadir at night. The regulation of the HPA axis also depends on the occupation of corticosteroid receptors. There are two types of corticosteroid receptors; the mineralocorticoid receptors (MR), which are mainly distributed in the hippocampus and the glucocorticoid (GR) receptors, which are distributed in other areas of the brain, such as the prefrontal cortex and the entorhinal cortex [4;5]. MR receptors are occupied when glucocorticoid levels are low to moderate, for instance in the evening, whereas GR receptors are only occupied when glucocorticoid levels are higher, as is usually the case in the morning and during stressful circumstances[6].

Elevated glucocorticoids are essential for the redistribution of energy resources, but the long-term elevation of glucocorticoids starves some tissues of necessary resources and hinders immune function, increasing the susceptibility to disease. While the stress response is critical for the successful adaptation to a real or perceived threat, a persistent stress response can be harmful. A critical feature of this stress response is that it terminates itself when the stressor has ended or is no longer perceived as a threat. The termination of the stress response is critical because the continued activation of the SNS and the HPA axis can be detrimental to the body. A racing heart and high blood pressure triggered by adrenaline to maximize blood flow is critical for assisting in escaping the stressor, but a persistent racing heart and high blood pressure increases the risk of heart failure and arteriosclerosis. [7;8]

Alterations in HPA axis functioning have also frequently been found in neuropsychiatric disorders. For instance, in Alzheimer's disease (AD) elevated levels of cortisol[9-12] are quite common and depression [13-17]and post-traumatic stress disorder [18-20]have both been associated with lowered and elevated levels of cortisol.

Aim of this thesis

The first aim of this thesis was to investigate the relations between stress, depression and early symptoms of Alzheimer's disease (AD), like cognitive decline and volume loss of the hippocampus and entorhinal cortex. The second aim was to investigate to what extent these relations were explained by the glucocorticoid cascade hypothesis (see figure 1, in chapter 1).In short, this hypothesis states that chronic exposure to glucocorticoids can lead to hippocampal volume loss, which then may result in a diminished inhibitory feedback to the HPA axis [21] This impaired inhibitory effect may result in hypersecretion of cortisol, which, in turn, may result in further neuronal loss of the hippocampus (figure 1, chapter 1).[22] Changes in HPA axis activity, hypercortisolemia, hippocampal volume loss and memory impairment are the common symptomatic cluster present in AD and depression. Because depression is common in AD (up to 50 % of patients suffer from depression) it has been hypothesized that depression and stress can lead to AD, via chronic exposure to elevated levels of cortisol.
In this thesis the following elements of the glucocorticoid cascade hypothesis were investigated and in the next paragraphs each of these elements is discussed in relation to findings from this thesis:

1) Chronic stress and stress-related disorders lead to long-term alterations in HPA axis activity and predominantly hyperactivation.
2) High levels of cortisol are associated with cognitive impairment and on the long-term will lead to cognitive decline
3) High levels of cortisol increase the risk for brain volume loss, particularly of the hippocampus.
4) Chronic stress and stress-related disorders are associated with cognitive impairment, cognitive impairment and brain volume loss and this association is explained by hyperactivity of the HPA axis.

1) Stress and long-term alteration in HPA axis activity

The experience of stress will lead to activation of the HPA axis and thus to higher levels of cortisol and in some cases stressors may lead to long-term changes in HPA axis regulation and according to the glucocorticoid cascade hypothesis could then lead to brain atrophy and cognitive decline. In chapter 2.1 and 3.3 we observed that the experience of life events may lead to long-term changes in HPA axis regulation. We found a differential effect of adverse life events experienced during childhood and adverse life events experienced later in life on morning cortisol. Adverse childhood events were associated with lower morning cortisol levels, whereas recently experienced

adverse life events were associated with higher levels of morning cortisol. We found no effect of the combination of childhood and recent events, although we expected that particularly persons who had experienced adverse events both in childhood and later in life would show the largest alteration in HPA axis activity. Possibly the observed differential effect cancelled out an interaction effect.

Interestingly, we found comparable findings within two different cohort studies (e.g. LASA and SMART-Medea), which suggests that this is a rather robust effect which is not influenced by differences in study populations nor differences in cortisol samples and life events assessment.

Thus, stressors may lead to hyperactivation but also to hypoactivation of the HPA axis, depending on when the stressor occurred. Possibly childhood events initially lead to hyperactiviation of the HPA axis and if the hyperactivity becomes chronic lead to hypo-activation. [23;24]. The exact mechanism by which this occurs is still unclear. A possible mechanism may be that long-lasting excessive secretion of cortisol leads to negative feedback inhibition of the pituitary to the adrenal cortex [25;26].

Differences in cortisol responses to stress may also be explained by personality, as the interpretation of a stressor depends largely on personality characteristics of an individual. In chapter 2.1 we however described that there was no main effect of the personality characteristics mastery, self-esteem and neuroticism on cortisol levels, although we found an interaction between age and neuroticism on evening cortisol, suggesting that younger persons with relatively high neuroticism levels have higher cortisol levels in the evening. Thus in this population of older persons we found only modest evidence for a direct relation between personality characteristics and basal HPA axis activity in older persons. So far previous studies on the relation between personality and HPA axis activity were performed in younger adults (age < 65 years) and their results were highly variable; associations between several personality characteristics and cortisol measures are found [27-31] So it seems that personality characteristics play a role in the regulation of the HPA axis in younger adults (age < 65 years), but that this effect is rather unspecific. Probably, personality characteristics play a role in the cortisol response to stressors and less in the regulation of basal HPA axis activity, as was suggested in a stress-induced study. [32]

2) HPA axis activity and cognition

In older persons (LASA), we found that high cortisol levels were associated with poorer memory performance, but not with memory decline (chapters 3.1 and 3.2), irrespective of cortisol sample (e.g. saliva or serum). We also found an interaction between cortisol levels and apolipoprotein (APOE) e4 genotype; in persons carrying the APOE-e4, we found that low morning cortisol, high levels of evening cortisol and flattened diurnal variability of cortisol were associated with memory decline, but not in non-carriers (chapter 3.2).

However, in patients with manifest arterial disease (SMART-Medea) we found no(cross-sectional) relation between cortisol levels and cognitive functioning (chapter 3.3). This null finding was unexpected, as both HPA axis activity and cognitive functioning were measured in great detail. It could be that patients with manifest arterial disease have altered HPA axis function and that we therefore did not find an association between cortisol levels and cognitive functioning. This seems however unlikely as cortisol levels were equal to cortisol levels measured in healthy subjects. Another possibility is that cortisol levels are only associated with cognitive dysfunction and decline in more vulnerable persons; for instance the very old, [33;34] persons with a specific genetic predisposition [35] or persons with mild cognitive impairment. [36;37] Participants within the SMART study may be in better health than is generally expected of patients with manifest arterial disease. This is a common phenomenon in cohort studies, as the severely ill, depressed and very old usually do not participate in follow-up measurements.

Our results suggest that particularly low diurnal variability of cortisol (thus low cortisol levels in the morning and high cortisol levels in the evening) are associated with poorer cognitive performance in older persons and with memory decline in APOE-e4 allele carriers. Possibly, APOE-e4 allele and aging both lead to alterations in HPA axis activity and that this subsequently leads to cognitive decline. This explanation is partially in favour of the glucoccorticoid cascade hypothesis, as with increasing age, loss of hippocampal neurons may result in a diminished inhibitory feedback to the HPA axis and could thus also lead to cognitive impairments. Nevertheless, another explanation for our findings could be that the interaction between cortisol and APOE-e4 allele represents an early stage of AD, as APOE-e4 allele is the most consistent genetic risk factor for AD and alterations in HPA axis activity have also been frequently found in AD. In the latter case HPA-axis alterations are merely a side-effect in stead of the cause of cognitive decline.

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3) HPA axis and the brain

In chapter 4.1 we reported that higher evening levels of cortisol and higher levels at awakening after ingesting 0.5 mg dexamethasone were associated with smaller hippocampal volumes. These results are in line with studies which indicated that the dexamethasone suppression test (DST) particularly depends on the negative feedback of the hippocampus and that high levels after DST are a result of hippocampal volume loss. [38;39] The level of evening cortisol also depends largely on the hippocampus, because the mineralocorticoid receptor is mainly distributed in the hippocampus and it is thought that this receptor is involved in the regulation of the evening nadir. [40]Consequently, hippocampal volume loss can result in a decrease of density in mineralocorticoid receptors and therefore an increase in evening levels.[41] However, since this was a cross-sectional study it is difficult to infer cause from consequence. Smaller hippocampal volumes may be a result of elevated cortisol levels, as is stated by

the glucocorticoid cascade hypothesis, [42] but our results can also represent the reverse causality; namely that high levels of cortisol are a result of smaller hippocampal volumes. This has been suggested in relation to the cortisol awakening response (CAR); it was shown that patients with hippocampal lesions showed a flattened CAR. In our study, we however found no association between hippocampal volume and morning cortisol levels.

In chapter 4.2 we describe a moderate relation between HPA axis activity and entorhinal cortex volume; with high evening cortisol being associated with smaller left entorhinal cortex volume, but not with right entorhinal cortex volume. Until now, the relation between HPA axis activity and entorhinal cortex has hardly been investigated, so it is difficult to explain these findings. It seems however that the entorhinal cortex, compared to the hippocampus, plays only a modest role in HPA axis regulation.

4) Stress, cognition and the brain

In chapter 3.3 we investigated whether life events experienced during childhood and later in life were associated with cognitive functioning and whether this association could be explained by HPA axis activity. Unexpectedly, we found that a history of childhood abuse was associated with better cognitive functioning. Also, recent events were associated with better executive functioning. Although, we found differential associations of childhood events and recent events with morning cortisol, HPA axis activity did not explain the relation between stressful life events and cognitive functioning.

A beneficial effect of a history of childhood abuse is contrary to general beliefs in psychiatry research, however recently animal studies have shown comparable findings. [43;44] They found that rodents who had received poor maternal care could cope better with stressful situations later in life than rodents who had received good maternal care. In humans, resilience after childhood adversities is also quite common [45]

Other studies on the relation between stressful experiences and risk for cognitive impairment and decline reported inconsistent findings. [46-48] There are large inter-individual differences in how persons cope with stress and it is likely that only in a small proportion the experience of life events leads to detrimental outcomes, like post-traumatic stress disorder, depression and cognitive impairment.

In the last chapter of this thesis we investigated whether depression is related to hippocampal and entorhinal cortex volume loss and whether this relation could be explained by HPA axis activity (chapter 4.2). Contrary to previous studies, we found that major depressive disorder was not associated with hippocampal or entorhinal cortex volumes. [49;50] However, we found a differential association of age of onset of depression with hippocampal or entorhinal cortex volumes; late-onset depression was associated with smaller entorhinal cortex volumes, whereas early-onset depression was associated with smaller hippocampal volumes. But this association was not explained by HPA axis activity.

It should be noted that we only found a modest reduction of hippocampal volume in early-onset depression (1.9 – 2.3 %), whereas we found considerable entorhinal cortex volume loss in late-onset depression (4.2 – 6.2 %). According to the glucocorticoid cascade hypothesis it would have been expected that early-onset depression is associated with more brain volume loss, as early-onset of depression is associated with recurrent depressive episodes and stress-related neurotoxic factors associated with repeated episodes of depression would lead to brain volume loss. Our findings do not agree with this and consequently, it could be that there is another mechanism underlying the relation between stress-related disorders, brain atrophy and cognitive decline.

Alternative hypotheses

We propose alternatives to the glucocorticoid cascade hypothesis to explain the results found in this thesis, which are described below and are also illustrated in Figure 1.

A) A mechanism other than alterations in HPA axis activity and chronic exposure to glucocorticoids may account for cognitive decline and brain atrophy in depression or after psychosocial stress. One often proposed explanation between stress, depression, brain atrophy and cognitive decline is vascular pathology. It has widely been recognized that there is an association between depression and cardiovascular diseases (CVD) [51;52]. A recent review indicates that 17-27 % of patients with CVD suffer from major depressive disorder (MDD) [53]. And several lines of evidence suggest that psychosocial stress, including life events and childhood trauma, can be a risk factor for CVD as well. [54-56]

Particularly late-onset depression has been associated with vascular brain pathology (e.g. white matter lesions) [57-59] In this thesis however we found that the association between late-onset depression and smaller entorhinal cortex volume was independent of white matter lesions and vascular risk factors. Also, it is unlikely that vascular pathology accounts for the relation between early-onset depression and brain volume loss.

Other mechanisms which have both been associated with brain volume loss and depression are dysregulations in calcium metabolism and glutamergic excitotoxicity. [60-64] For instance it is thought that excessive glutamate action, especially extrasynaptic glutamate, may be deleterious for neuronal function [65] and this may explain the relation between depression and also in Alzheimer's disease. However since glutamate and calcium are involved in many neurological and psychiatric disorders it seems unlikely that they could specifically account for the relation between stress-related disorders and cognitive decline and brain atrophy.

B) Another explanation may be that psychosocial stress and depression interact with a mechanism or vulnerability factor and that it will then lead to cognitive decline and

brain atrophy. This would explain the heterogeneity within depression, as not everyone who is depressed shows cognitive impairment and/or will show cognitive decline and brain atrophy. For instance, it has been suggested that depression interacts with apolipoprotein (APOE) e4 allele on the risk for Alzheimer's disease; carriers of APOE-e4 allele have increased risk for AD when they are also depressed. [66-68] Our finding that only in APOE-e4 carriers alterations in HPA axis activity lead to cognitive decline are also in line with this explanation, as alterations in HPA axis activity have frequently been found in relation to depression. [17;69]

C) Brain volume loss, depression and HPA axis dysregulation may be epiphenomena of an unknown process and may or may not have a direct relationship to one another. A good candidate for this unknown process is Alzheimer's disease (AD). AD has been associated with HPA axis dysregulation[9;10] and depression [70-73]

Cardiovascular diseases are associated with HPA-axis dysregulation and it has also been suggested that the relation between depression and cardiovascular diseases could be the other way around; depression being a consequence of cardiovascular disease.[52;74] Cardiovascular diseases are also a risk factor for brain atrophy [75;76] and cognitive decline, [77;78] independent of depression. Thus cardiovascular diseases may also give rise to brain atrophy, HPA-axis dysregulation and depression. This would primarily be an explanation for the relation between late-onset depression and cognitive decline and brain atrophy and not for early-onset of depression. In this thesis, we however found that the association between smaller entorhinal cortex volume and late-onset depression was independent of vascular risk factors. The entorhinal cortex is thought to be less sensitive to vascular pathology than the hippocampus, [79] thus our findings do not necessarily rule out this explanation.

Another factor which could give rise to brain atrophy, HPA-axis dysregulation and depression may be a certain genetic predisposition. Neuropsychiatric disorders are however heterogeneous, so it seems unlikely that one genetic variation might explain the aetiology of stress-related disorders, cognitive decline and brain atrophy.

D) Smaller brain volumes lead to depression and other stress-related disorders and also to cognitive impairment and cognitive decline. A reverse causality has been proposed before and has also been shown in post-traumatic stress disorder. [80] Longitudinal studies have also shown that a smaller hippocampal volume predicts poorer clinical outcome, which would also be indicative for a higher vulnerability marked by smaller hippocampal volumes. [81;82] In line with this, impaired neurogenesis in the hippocampus has been suggested to play a causal role in neuropsychiatric disorders, like depression and Alzheimer's disease and that this also explains the cognitive deficits as seen in these disorders (e.g. memory impairments). [83;84]

Conclusions

The findings of these thesis suggest that particularly late-onset depression is associated with brain volume loss, which could be indicative of early symptoms of Alzheimer's disease, whereas early-onset depression was only associated with moderate brain volume loss. We hypothesize that depression does not lead to brain volume loss; rather we think that late-onset depression and brain atrophy are both a result of a common causal factor, for instance Alzheimer's disease. In case of early-onset depression, it is likely that smaller hippocampal volumes precede the onset of depression and could even be a risk factor for the onset of depression. Furthermore, we found no evidence for stress-related cognitive impairment, as the experience of life events was associated with better cognitive functioning.

The relations between depression and brain atrophy and stress and cognitive functioning were not explained by HPA axis activity. Also, we found that not only elevated levels of cortisol, but also lower levels of cortisol and flattened diurnal variability are associated with psychosocial stress (e.g. life events) and cognitive decline. Thus, our findings together with those from several previous studies strengthen the argument against the glucocorticoid cascade hypothesis.

Nevertheless, some of our findings fit the hypothesis. For instance, we found that elevated levels of cortisol were associated with smaller hippocampal and entorhinal cortex volumes and alterations in HPA axis regulation were associated with stress, depression and cognitive decline. Even though these alterations also incorporated lower levels of cortisol these findings are at least partially in line with the glucocorticoid cascade hypothesis. As a consequence, the findings of this thesis are not sufficient for conclusive disagreement or confirmation of the glucocorticoid cascade hypothesis.

Methodological issues

There are some methodological issues which should be considered and which have not been discussed in the previous chapters. In this thesis data were used from two large cohort studies. These cohort studies had each their own particular domain, namely older persons and patients with manifest arterial disease. We thus do not know if the observed associations can be generalized to the younger and/or more healthy population. Since increasing age and cardiovascular diseases are also associated with increased risk for cognitive decline and brain atrophy,[8;85-87], one would expect that older persons and persons with manifest arterial disease are more vulnerable to the possible detrimental effects of stress and depression on cognitive functioning and the brain. Therefore, examining the relation between stress-related disorders, HPA-axis activity and cognitive decline and brain volumes in these populations may have made it more likely to find an association that may not have been observed in younger and healthier populations. Another advantage of investigating these two populations is that we could replicate some of our findings, which increases the internal validity of

some of our findings.

It is questionable to which extent results found in the LASA study are comparable to results found in the SMART-Medea study. In both studies we investigated the relation between childhood and recent events and salivary cortisol levels and despite differences in methodologies used we found comparable associations. We also investigated the cross-sectional relation between cortisol levels and cognitive functioning in both cohort studies, but this did not result in comparable findings. It remains unclear whether this discrepancy in findings is due to methodological differences or due to differences in populations used. Or possibly the effect of cortisol on cognition is less robust than the relation between childhood and recent events and cortisol levels.

Moreover, measurements of cortisol were not identical within and between the cohort studies. In LASA cortisol was measured in blood serum, collected in the morning before 10 am and in saliva, collected within 30 minutes after awakening and in the evening before going to bed. Whereas, in the SMART-Medea study cortisol was measured in saliva, using 7 samples of saliva over a period of 24 hours to obtain the circadian rhythm. Cortisol levels measured in saliva and blood serum highly correlate and are both considered to be reliable and valid measurement methodologies. [88] As a result we do not think that the differences in results can be explained by the differences in cortisol sampling.

Moreover, there is some debate on whether cortisol can be considered to be a good proxy for HPA axis activity, according to some studies it is [89;90], whereas it has also been suggested that other neuroendocrine measures, such as adrenocorticotropin (ACTH), or the ratio between dehydroepiandrosterone (DHEA) and cortisol concentrations may be more sensitive to pick up the effects of neuropsychiatric disorders on HPA axis regulation than only cortisol measurements. [91-93] It would therefore be of interest to study other neuroendocrine measures as well in relation to cognitive functioning and brain volume.

The two cohort studies used in this thesis differ on many other aspects. One of which is the measurement of cognitive functioning. Cognitive functioning was measured using a fairly limited test battery; which assessed global cognitive functioning, verbal memory and processing speed and attention with only three tests in LASA. Whereas, in the SMART-Medea study cognitive functioning was determined using an extensive neuropsychological test battery. In the SMART-Medea study it was therefore possible to assess the domains of memory performance, executive functioning and processing speed and attention in great detail. It should therefore have been possible to detect more subtle impairments in the SMART-Medea study than it was in LASA. This discrepancy makes it even more surprising that we did not find an association between cortisol levels and cognitive functioning in SMART.

The assessment of life events was also done in more detail in the SMART-Medea study than in LASA. In LASA recent events were assessed over the foregoing three years

and childhood life events were assessed using an open-ended question. Limitations of these methods are that the recent events could not be classified as being recent and because of the open-ended question on childhood events we probably missed certain important childhood events. In the SMART-Medea study, questionnaires were used to assess childhood and recent life events as well, but the questionnaire on recent life events was focused on events which happened only within the preceding year and structured questionnaires were used to assess childhood events, which decreased the likelihood of under-reporting.

Another major discrepancy between the SMART-Medea study and LASA is the study design. Even though we used follow-up data of the SMART-Medea study, many of the variables used were not available at baseline, whereas in LASA, data were available for the majority of variables at baseline and several follow-up measurements, making it possible to study the relations longitudinally. A cross-sectional design makes it impossible to discern cause from consequence (chapters 3.3, 4.1 and 4.2). Longitudinal studies are therefore needed to further unravel the relation between stress, depression, HPA axis regulation and early symptoms of Alzheimer's disease.

Future studies

Within the SMART-Medea study a great deal of data are still available; making it possible to further investigate how stress and depression affect the brain and cognitive functioning. For instance, the relation between life events and hippocampal and entorhinal cortex volume could also be investigated. It would be interesting to see whether the beneficial effect of stress on cognitive functioning could be replicated in a study on brain volumes.

Ideally, to investigate whether there is a causal relation between stress, depression and brain atrophy, brain volumes are measured before and after the onset of depression and/or experience of severe stress. This can be done in specific populations, who are at great risk for developing depression and/or experiencing severe stress. For instance, one could assess brain volumes and cognitive functioning in soldiers before, during and after they have been sent out on a military mission. Preferably, these soldiers should be followed-up for a long time, to measure the long-term effects of stress on the brain.

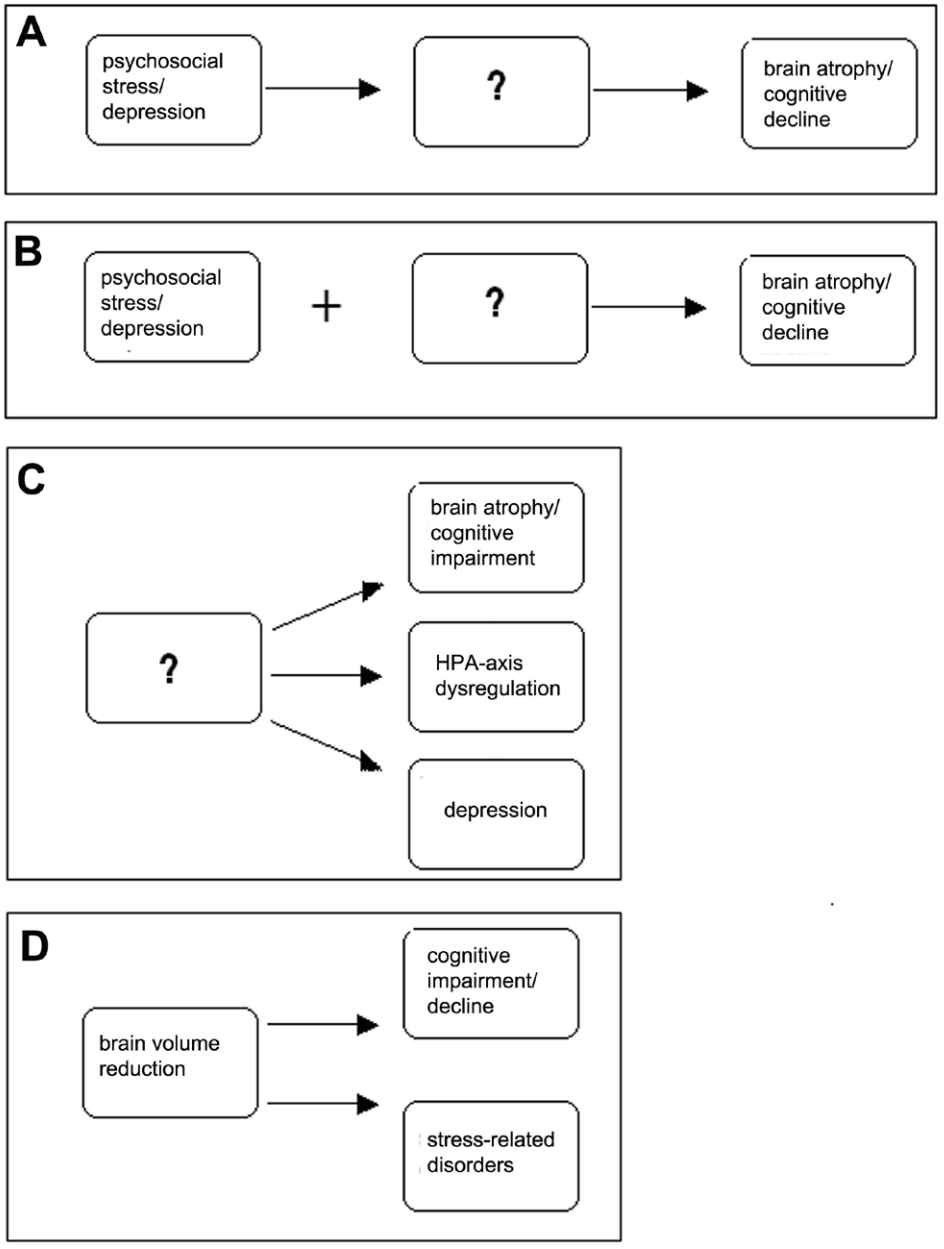
Another population in which the causal relation between stress-related disorders, brain atrophy and cognitive decline could also be investigated is monozygotic twins. Differences between monozygotic twins can only be explained by differences in environmental factors and thus they are an ideal population to study the effects of stress-related disorders and whether alterations in HPA axis activity are a result of these disorders.

Long-term changes in HPA axis activity can be determined by measuring basal cortisol levels as was done in the studies described in this thesis. But, it could also be that stress-related disorders alter the way the HPA axis is activated by new

stressors. Therefore, future studies should also determine whether cortisol responses to new stressors are related to cognitive decline and brain atrophy. This can be done in experimental settings, for instance by a public speaking task or mental arithmetic challenges with social evaluations. A limitation of this design is that the setting is not comparable to real life situations and the experimental stressor is only a moderate stressor and will thus not elicit a true stress response in everyone. Another possibility would therefore be to ask participants to collect saliva directly after experiencing a stressor in real life. This could for instance be implemented in a study among combat soldiers. Whether participants will be able to collect saliva directly after a severe event is however questionable.

At last, in this thesis we did not study patients with Alzheimer's disease, whereas we were interested in the relation between stress, depression and early symptoms of Alzheimer's disease. Future studies among older persons with and without Alzheimer's disease should therefore study whether HPA axis dysregulation explains the relation between (chronic) stress, depression and onset of Alzheimer's disease.

Figure 1. Schematic representation of alternative hypotheses



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Chapter 6

Summary
Nederlandse samenvatting
Dankwoord
Curriculum Vitae

6

Summary

Alzheimer's disease (AD) is the most common cause of dementia, and is characterized by cognitive decline, medial temporal lobe atrophy and various behavioural problems. Also, 30 to 50 % of patients with AD suffer from depression, leading to more functional disability, worsening of cognitive deficits and extra burden for the caregiver. The exact nature of this co-occurence remains however unclear. One possible explanation is based on the glucocorticoid cascade hypothesis that states that depression leads to dysregulation in the hypothalamic-pituitary-adrenal (HPA) axis and consequently to increased levels of glucocorticoids. The HPA-axis gets activated by stress and the end product of this activation are glucocorticoids, in humans the main glucocorticoid is cortisol. Chronic exposure to excessive glucocorticoids would lead to damage to the hippocampus and eventually lead to dementia. In this thesis we therefore investigated whether depression and stress are related to early symptoms or preclinical markers of AD, like cognitive decline and volume loss of medial temporal lobe structures such as the hippocampus and entorhinal cortex, and to which extent these relations are explained by HPA-axis activity.

Data were used from two large cohort studies, the Longitudinal Aging Study Amsterdam (LASA) and the Second Manifestations of ARTerial disease-Memory, depression and aging (SMART-Medea) study.

Psychosocial determinants of HPA-axis activity.

We first investigated to which extent personality characteristics were related to HPA-axis regulation in older persons (**chapter 2.1)**. The study sample consisted of 1,150 participants (mean age 74 ± 7 years; 48% male) from LASA. The results showed that overall the personality characteristics mastery and self-esteem were not associated with HPA - axisactivity as measured with salivary awakening and evening cortisol. However, we did find an interaction between age and neuroticism on evening cortisol, suggesting that high neuroticism was associated with elevated levels of evening cortisol in the younger old, but not in the older old.

It has been hypothesized that stressful life events increase the susceptibility to psychiatric disorders through long-term alterations in HPA- axis regulation. Therefore we investigated both in LASA (1,055 participants, mean age 74 ± 7 years ; 47% male) (**chapter 2.2)** and in the SMART-Medea study (736 patients, mean age 62 ± 9 years; 81% male) (**chapter 3.3)** whether adverse life events experienced during childhood and adverse life events experienced later in life were associated with HPA-axis regulation. Despite differences in assessments of life events and HPA-axis activity, we found a differential effect of early and late life events on morning cortisol in both studies. Adverse childhood events were associated with lower morning cortisol levels, whereas recently experienced adverse life events were associated with higher levels of morning cortisol.

These comparable findings suggest that the long-term alterations in HPA-axis activity after the experience of life events is a rather robust effect, which is not influenced by differences in study populations or differences in cortisol samples and life events assessments.

Stress, HPA-axis and cognition

It has been hypothesized that chronic exposure to the stress hormone cortisol has a damaging effect on the brain and cognitive functioning. In LASA, we investigated whether elevated cortisol levels were associated with cognitive impairment and cognitive decline. In **chapter 3.1** (1,154 participants, mean age 75.1 ± 6.5 years ; 48% male) we investigated whether serum cortisol levels, collected in the morning, were associated with cognitive performance and cognitive decline and whether this association differed by age, sex, and depression status. We found that high levels of free cortisol were associated with poorer performance on verbal learning (B= -0.32; 95%CI: -0.64 to -0.01). Furthermore, in women, but not in men, high levels of free cortisol (B=-0.85; 95 % CI: -1.40;-0.31) were associated with slower speed of information processing. The associations between cortisol and cognitive performance were the same for the younger and the older old, and for depressed and non-depressed persons. Higher levels of cortisol were not associated with cognitive decline over a period of six years.

In **chapter 3.2** (991 participants, 74.5±7.2 years; 46.4% male) we determined whether salivary cortisol , collected after awakening and in the evening, was associated with cognitive decline and whether this association was modified by the apolipoprotein (APOE)-e4 allele.

High evening levels of cortisol and low diurnal variability of cortisol were associated with poorer delayed recall (B=-0.20; 95% CI: -0.37 to -0.03 and B = 0.54; 95% CI: 0.11 to 0.99, respectively) and poorer memory retention (B= -1.55; 95% CI: -2.81 to -0.28 and B = 4.15; 95% CI: 0.44 to 7.87, respectively). But no associations were found with verbal learning, global cognitive functioning nor with information processing speed, nor were cortisol levels associated with cognitive decline over a period of four years. However, in APOE-e4 carriers, lower levels of morning cortisol (B= 0.14; 95% CI: 0.02 to 0.25) and flattened diurnal variability of cortisol (B = 0.17; 95% CI: 0.06 to 0.30) were associated with decline on verbal learning, but only in APOE-e4 carriers. Also, in APOE-e4 carriers higher levels of evening cortisol and flattened diurnal variability of cortisol were associated with decline on delayed recall. No relation between cortisol and cognitive decline was found in the APOE- e4 non-carriers.

Thus, in both studies within the LASA study we found that there was a relation between high levels of cortisol and poorer cognitive functioning, but that only in APOE-e4 carriers, HPA-axis activity was associated with cognitive decline. From these findings, we hypothesize that the APOE-e4 allele and aging both lead to alterations in HPA axis activity and that this subsequently leads to cognitive decline.

Because stressful life events may lead to long-term changes in HPA-axis activity and stressful life events have also been associated with cognitive impairment

we sought to investigate the relation between childhood and recent life events, HPA-axis activity and cognitive functioning in **chapter 3.3**. The study sample consisted of 736 participants (mean age 62 ± 9 years; 81% male) from the SMART-Medea study. Surprisingly, participants with childhood abuse had significantly better memory performance and better processing speed/attention than participants without childhood abuse. Childhood events, such as divorce of parents, were not associated with cognitive functioning. Participants who experienced recent life events had better executive functioning. HPA-axis activity was not associated with cognitive functioning, and could therefore not explain the relation between stressors and cognitive functioning. Possibly, HPA-axis activity was not associated with cognitive functioning in this study, because other mechanisms (like cardiovascular disease) influenced the relation greatly or because the participants were less old than in the LASA study.

Depression, HPA-axis and the brain

It has often been hypothesized that high levels of cortisol can have a detrimental effect on the hippocampus, because the hippocampus is thought to play an inhibitive role in the regulation of the HPA-axis. In **chapter 4.1** we examined the direct relation between hippocampal volumes and HPA- axis activity. Within the SMART-Medea study, 575 patients (mean age 62±9 years; 81 % male) the diurnal cortisol rhythm was assessed and volumetric measurements of the hippocampus were performed. Higher evening levels and higher awakening levels after dexamethasone were associated with smaller hippocampal volumes (B per SD (4.2) increase= -0.09 ml; 95%CI -0.15 to -0.03 ml and B per SD (2.5) increase = -0.07 ml; 95%CI -0.13 to -0.01 ml, respectively). The awakening response was not significantly associated with hippocampal volumes. In this study we thus confirmed that the hippocampus is sensitive to high levels of cortisol.

Structural brain changes, such as hippocampal volume loss, have often been found in major depressive disorder (MDD) and MDD has been associated with changes in HPA-axis activity. It is therefore thought that HPA-axis activity may explain the relation between MDD and structural brain changes. In **chapter 4.2** we investigated the association of MDD and depressive episodes in the past with hippocampal and entorhinal cortex volumes, and whether HPA-axis activity explained this association. Within the SMART-Medea study (636 participants, mean age 62 ± 9 years, 81% male) MDD was not associated with hippocampal or entorhinal cortex volumes. However we did find a differential relation of age of onset of depression on hippocampal and entorhinal cortex volume; participants with early-onset depression (first depressive episode before age of 50 years) had slightly but statistically significantly smaller hippocampal volume than those who had never been depressed, whereas participants with late-onset depression (first depressive episode after age of 50 years) had about 5% smaller entorhinal cortex volume. HPA-axis activity did however not explain these differences.

In **chapter 5** we discussed the findings of this thesis within the framework of the glucocorticoid cascade hypothesis and propose some alternative explanations for the relation between stress, depression and early symptoms of Alzheimer's disease.

The findings of these thesis suggest stress and depression are associated with alterations in HPA-axis activity, but that HPA-axis activity cannot explain the relation between stress, depression and early symptoms of Alzheimer's disease. Furthermore, we hypothesize that depression first occurring later in life is not a risk factor for brain atrophy and Alzheimer's disease; rather we think that late-onset depression and brain atrophy both result from incipient Alzheimer's disease or that brain atrophy leads to depression. With respect to the findings in early-onset depression, it is more likely that smaller hippocampal volumes precedes the onset of depression; thus suggesting that it could be a susceptibility to develop depression. Furthermore, we found no evidence for stress-related cognitive impairment, as the experience of life events was associated with better cognitive functioning.

Future studies should make use from the data available within the SMART-Medea study, making it possible to further investigate to what extent stress and depression affect the brain and cognitive functioning. Also, longitudinal studies among older persons with and without Alzheimer's disease are needed to improve our understanding of the relation between (chronic) stress, depression and onset of Alzheimer's disease.

Nederlandse samenvatting

De ziekte van Alzheimer is de meest voorkomende variant van dementie en wordt gekenmerkt door cognitieve achteruitgang, atrofie van de mediaal temporaal kwab en een groot aantal gedragsstoornissen. Bovendien lijdt 30 tot 50 procent van de patiënten met de ziekte van Alzheimer aan een depressie, hetgeen als gevolg kan hebben dat de cognitieve problemen en functionele stoornissen verergeren. Het is nog onduidelijk of depressie een vroeg symptoom is van neurodegeneratie, of het een psychologische reactie is op de cognitieve achteruitgang of dat depressie een risico factor is voor het ontstaan van de ziekte van Alzheimer. Een mogelijke verklaring is gebaseerd op de glucocorticoid cascade hypothese.

Zodra een situatie wordt geïnterpreteerd als mogelijke bedreiging wordt de hypothalamus-hypofyse-bijnierschors (HHB) as geactiveerd. De bijnierschors scheidt glucocorticoiden af en in mensen is cortisol het meest voorkomende glucocorticoid. Volgens de glucocorticoid cascade hypothese, leidt chronische blootstelling aan verhoogd cortisol tot atrofie van de hippocampus en dit zou leiden tot verminderde inhibitie van de HHB-as, met als gevolg verhoogde niveaus van cortisol. De hippocampus speelt een belangrijke rol in het opslaan en terughalen van informatie en is een van de eerste structuren die wordt aangetast door de ziekte van Alzheimer. Net als de ziekte van Alzheimer, is depressie ook geassocieerd met hippocampus atrofie en verminderde geheugenfuncties. Bovendien is verhoogd cortisol en HHB-as disregulatie een veel voorkomend kenmerk van depressie. Daarom wordt verondersteld dat depressie en stress een risicofactor kunnen zijn voor de ziekte van Alzheimer, via chronische blootstelling aan verhoogde cortisol niveaus. In dit proefschrift hebben we daarom onderzocht in welke mate psychosociale stress en depressie gerelateerd zijn aan vroege symptomen van de ziekte van Alzheimer, zoals cognitieve achteruitgang en atrofie aan hippocampus en entorhinale cortex, en in hoeverre deze relaties verklaard zouden kunnen worden door HHB-as activiteit.
Om dat te kunnen onderzoeken is gebruik gemaakt van data van twee grote cohort studies, namelijk de Longitudinal Aging Study Amsterdam (LASA) studie en de Second Manifestations of ARTerial disease-Memory, depression and aging (SMART-Medea) studie.

Psychosociale determinanten van HHB-as activiteit

In LASA hebben we eerst onderzocht of persoonlijkheidskenmerken geassocieerd zijn met HHB-as regulatie bij oudere personen (**hoofdstuk 2.1)**. De studiepopulatie bestond uit 1,150 participanten (gemiddelde leeftijd 74.8 ± 7.1 jaar; 48% mannelijk geslacht). De resultaten lieten zien dat de persoonlijkheidskenmerken 'mastery' en zelfverzekerdheid niet waren gerelateerd aan HHB-as regulatie, zoals gemeten in speeksel in de ochtend en laat in de avond. Echter, er bleek een interactie te zijn met leeftijd en neuroticisme op avond cortisol (B = -0.001; 95 % CI: -0.003 tot 0.00);

deze suggereert dat hoog neuroticisme is geassocieerd met hogere avondwaardes van cortisol in de jongere ouderen (leeftijd < 75 jaar) (B = 0.02; 95 % CI: 0.01 tot 0.03), maar niet in de oudere ouderen (leeftijd > 75 jaar). Deze studie laat zien dat persoonlijkheidskenmerken nauwelijks een rol spelen in HHB-as regulatie bij ouderen (leeftijd > 65 jaar).

Stressvolle gebeurtenissen, zoals seksueel misbruik, overlijden van een partner of werkloosheid, kunnen resulteren in veranderingen in HHB-as activiteit en dit zou de kwetsbaarheid voor psychiatrische aandoeningen vergroten. Daarom hebben we zowel in LASA (1,055 participanten, gemiddelde leeftijd 74.8 ± 7.1 jaar ; 47% mannelijk geslacht) (**hoofdstuk 2.2)** als in de SMART-Medea studie (736 patiënten, gemiddelde leeftijd 62±9 jaar; 81% mannelijk geslacht) (**hoofdstuk 3.3)** onderzocht of stressvolle gebeurtenissen vroeg en laat in het leven geassocieerd zijn met veranderingen in HHB-as activiteit. Ondanks grote verschillen in de manier waarop HHB-as activiteit en stressvolle gebeurtenissen werden gemeten, vonden we in beide studies een differentiële relatie van stressvolle gebeurtenissen tijdens de kindertijd en stressvolle gebeurtenissen later in het leven op ochtend cortisol. Stressvolle gebeurtenissen tijdens de kindertijd waren geassocieerd met lagere ochtendwaardes van (cortisol na ontwaken in LASA: B=-0.10; 95% CI: -0.17 tot -0.04; en AUCg ochtend cortisol in SMART-Medea: B= -1.56 nmol/L*uur; 95% CI: -2.83 tot -0.30), terwijl stressvolle gebeurtenissen later in het leven geassocieerd waren met hogere ochtendwaardes van cortisol (cortisol na ontwaken in LASA: B=0.10; 95% CI: 0.02 tot 0.18; en AUCg ochtend cortisol in SMART-Medea: B=1.31 nmol/L*uur; 95% CI: 0.01 tot 2.60).

Deze vergelijkbare bevindingen suggereren dat lange termijn veranderingen in HHB-as regulatie na stressvolle gebeurtenissen een tamelijk robuust effect is dat niet wordt beïnvloed door verschillen in studiepopulatie, noch door verschillen in meetmethodes voor stressvolle gebeurtenissen en HHB-as activiteit.

Stress, HHB-as en cognitie

Chronische blootstelling aan verhoogd cortisol kan een schadelijk effect hebben op het brein en cognitief functioneren. In LASA onderzochten wij of verhoogd cortisol samenhangt met cognitief disfunctioneren en cognitieve achteruitgang. In **hoofdstuk 3.1** (1,154 participanten, gemiddelde leeftijd 75.1 ± 6.5 jaar ; 48% mannelijk geslacht) werd onderzocht of serum cortisol, gemeten in bloed in de ochtend, geassocieerd was met cognitief functioneren en cognitieve achteruitgang en of dit beïnvloed werd door leeftijd, geslacht en depressie. Hogere niveaus van cortisol waren geassocieerd met verminderd verbaal geheugen (B= -0.32; 95%CI: -0.64 tot -0.01). Verder, vonden we dat bij vrouwen, maar niet bij mannen, hoog cortisol geassocieerd was met minder snelle informatieverwerking (B=-0.85; 95 % CI: -1.40;-0.31). Er was geen effect van leeftijd of depressie, noch was hoog cortisol gerelateerd aan meer cognitieve achteruitgang over een periode van zes jaar.

Vervolgens, in **hoofdstuk 3.2** (991 participanten, gemiddelde leeftijd 74.5±7.2 jaar; 46.4% mannelijk geslacht) onderzochten we of cortisol, gemeten in speeksel na ontwaken en in de avond, was geassocieerd met cognitieve achteruitgang en of deze relatie beïnvloed werd door de apolipoproteine (APOE)-e4 allele.
Hoog avondcortisol en vlak circadiaan ritme van cortisol waren geassocieerd met verminderde verbale recall (respectievelijk B=-0.20; 95% CI: -0.37 tot -0.03 and B = 0.54; 95% CI: 0.11 tot 0.99) en geheugen retentie (respectievelijk B= -1.55; 95% CI: -2.81 tot -0.28 and B = 4.15; 95% CI: 0.44 tot 7.87). We vonden echter geen relatie tussen cortisol en globaal cognitief functioneren en informatie verwerkingssnelheid, tevens waren cortisol niveaus niet gerelateerd aan meer cognitieve achteruitgang over een periode van vier jaar. Echter, in APOE-e4 dragers, laag ochtendcortisol (B= 0.14; 95% CI: 0.02 tot 0.25) en vlak circadiaan ritme van cortisol (B = 0.17; 95% CI: 0.06 tot 0.30) waren geassocieerd met achteruitgang in verbaal leervermogen, terwijl hoog avondcortisol (B= 0.09; 95% CI: 0.01 tot 0.17 en vlak circadiaan ritme van cortisol ((B= 0.05; 95 % CI: -0.01 tot 0.11) geassocieerd waren met achteruitgang in verbale recall. Geen relatie tussen cortisol en cognitieve achteruitgang werd gevonden in participanten zonder de APOE- e4 allele.

In beide studies vonden we dat bij ouderen hoog cortisol geassocieerd is met verminderd cognitief functioneren, maar alleen in participanten met een genetische kwetsbaarheid voor de ziekte van Alzheimer was cortisol geassocieerd met cognitieve achteruitgang. Mogelijk leiden de APOE-e4 allele en veroudering allebei tot veranderingen in HHB-as regulatie met cognitieve achteruitgang als gevolg.

Stressvolle gebeurtenissen kunnen leiden tot lange termijn veranderingen in HHB-as regulatie en cognitief disfunctioneren. Daarom onderzochten we in **hoofdstuk 3.3** de relatie tussen stressvolle gebeurtenissen tijdens de kindertijd en in afgelopen jaar, HHB-as regulatie en cognitief functioneren. Voor deze studie werd gebruikt gemaakt van de SMART-Medea studie (736 participanten, gemiddelde leeftijd 62±9 jaar; 81% mannelijk geslacht) Tegen onze verwachting in vonden we dat participanten met een geschiedenis van kindermisbruik beter scoorden op geheugenfuncties (gemiddeld verschil=0.16; 95% CI: 0.00-0.32) en informatieverwerking (gemiddeld verschil =0.18; 95% CI: 0.03-0.34). Overige stressvolle gebeurtenissen uit de kindertijd waren niet geassocieerd met cognitief functioneren. Participanten die twee of meer stressvolle gebeurtenissen hadden meegemaakt in afgelopen jaar scoorden beter op executief functioneren (gemiddeld verschil = 0.18; 95% CI: 0.02 tot 0.35). In tegenstelling tot de bevindingen in LASA, was in deze studie HHB-as activiteit niet geassocieerd met cognitief functioneren en verklaarde ook niet de relatie tussen stressvolle gebeurtenissen en cognitief functioneren.

Depressie, HHB-as en het brein

Omdat de hippocampus een inhiberende rol speelt in de regulatie van de HHB-as, wordt verondersteld dat verhoogd cortisol met name een schadelijk effect heeft op de hippocampus. In **hoofdstuk 4.1** hebben we daarom de directe relatie tussen HHB-as activiteit en hippocampus volume onderzocht. In de SMART-Medea studie werd bij 575 patienten (gemiddelde leeftijd 62±9 jaar; 81 % mannelijk geslacht) het circadiane ritme van cortisol en hippocampus volume gemeten. Hoog avondcortisol en verhoogd ochtendcortisol na de dexamethason suppressie test waren geassocieerd met kleiner hippocampaal volume (respectievelijk B per SD (4.2) stijging= -0.09 ml; 95%CI -0.15 tot -0.03 ml and B per SD (2.5) stijging = -0.07 ml; 95%CI -0.13 tot -0.01 ml). Ochtendcortisol was niet gerelateerd aan hippocampus volume.

Structurele veranderingen in het brein worden regelmatig gevonden bij depressie en er wordt verondersteld dat dit verklaard kan worden door HHB-as activiteit. In **hoofdstuk 4.2** onderzochten we daarom de relatie tussen depressie in de afgelopen 12 maanden, depressieve episodes in de voorgeschiedenis en hippocampus en entorhinale cortex volume. Vervolgens gingen we na of deze relatie verklaard kon worden door HHB-as activiteit. De studie werd uitgevoerd in de SMART-Medea study (636 participanten, gemiddelde leeftijd 62±9 jaar, 81% mannelijk geslacht). We vonden geen verschil in hippocampus en entorhinale cortex volume tussen participanten met of zonder een depressie in de afgelopen 12 maanden. We vonden echter wel een differentiële relatie van de leeftijd waarop een eerste depressieve episode was doorgemaakt en hippocampus en entorhinale cortex volume; participanten met een eerste depressieve episode vóór de leeftijd van 50 jaar hadden een kleiner hippocampus volume (gemiddeld verschil: -0.06 ml; 95% CI -0.001 tot -0.123 ml), terwijl participanten met een eerste depressieve episode na de leeftijd van 50 jaar een kleiner entorhinale cortex volume hadden (gemiddeld verschil: -0.009 ml; 95% CI -0.001 tot -0.017 ml). Deze bevindingen konden niet worden verklaard door veranderingen in HHB-as activiteit.

In **hoofdstuk 5** werden de bevindingen van dit proefschrift besproken in relatie tot de glucocorticoid cascade hypothese en een aantal alternatieve verklaringen.

Op basis van de resultaten van dit proefschrift hypothetiseren wij dat de glucocorticoid cascade hypothese de relatie tussen psychosociale stress, depressie en vroege symptomen van de ziekte van Alzheimer niet volledig kan verklaren. Mogelijk leidt depressie niet tot breinatrofie, maar worden depressie en brein atrofie beide veroorzaakt door eenzelfde achterliggend mechanisme, zoals de ziekte van Alzheimer. Met name in het geval van depressie op latere leeftijd lijkt het aannemelijk dat dit een indicatie is voor een vroeg stadium van de ziekte van Alzheimer, omdat wordt verondersteld dat de entorhinale cortex nog eerder is aangetast dan de hippocampus bij de ziekte van Alzheimer. Terwijl een kleiner hippocampus volume een kwetsbaarheid zou kunnen zijn voor het ontstaan van een depressie op jongere

leeftijd. Verder vonden we in dit proefschrift geen bewijs dat cognitief disfunctioneren een gevolg kan zijn van stressvolle gebeurtenissen, omdat participanten die stressvolle gebeurtenissen rapporteerden juist verbeterde cognitieve capaciteiten hadden. Er blijven echter nog veel vragen onbeantwoord en het zou daarom interessant zijn om de relatie tussen psychosociale stress, depressie en vroege symptomen van de ziekte van Alzheimer in longitudinale studies te onderzoeken in ouderen met en zonder de ziekte van Alzheimer.

Curriculum Vitae

Lotte Gerritsen was born on August 13th, 1981, in Eindhoven, the Netherlands.
After graduating from grammar school at the Van Maerlant lyceum in Eindhoven in September 1999, she studied Psychology at the University of Amsterdam and graduated in September 2004. In September 2003 she started studying Neuroscience & Cognition at the Utrecht University and obtained her degree in October 2005. After graduation she worked as a clinical neuropsychologist at the mental health institute Altrecht at the department of elderly psychiatry and taught research courses at Utrecht University, department of Psychology.
In September 2006 she started working on the research described in this thesis at the Julius Center for Health Sciences and Primary care, University Medical Center Utrecht and the EMGO institute, VU Medical Center Amsterdam, under supervision of Prof. dr. Y van der Graaf, Prof. dr. B.W.J.H. Penninx, Dr. M.I. Geerlings and Dr. H.C. Comijs. She obtained her degree in Clinical Epidemiology at the Utrecht University in September 2009.
As of January 2010 she is working as a postdoctoral researcher at the Donders Center for Cognitive Neuroimaging of the Donders Institute for Brain, Cognition and Behaviour in Nijmegen.