

Depression, Hypothalamic Pituitary Adrenal Axis, and Hippocampal and Entorhinal Cortex Volumes—The SMART Medea Study

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Background: 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. We investigated the association of MDD and history of depression with hippocampal and entorhinal cortex volumes and whether HPA axis activity explained this association.

Methods: In 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, a 12-month diagnosis of MDD and history of depression were assessed. Age of first depressive episode was classified into early-onset depression (< 50 years) and late-onset depression (≥ 50 years). HPA axis regulation was assessed by four morning saliva samples, two evening samples, and one awakening sample after .5 mg dexamethasone. Hippocampus and entorhinal cortex volume were manually outlined on three-dimensional T1-weighted magnetic resonance images.

Results: General linear models adjusted for demographics, vascular risk, antidepressant use, and white matter lesions showed that ever having had MDD was associated with smaller hippocampal volumes but not with entorhinal cortex volumes. Remitted MDD was related to smaller entorhinal cortex volumes ($p < .05$). Participants with early-onset depression had smaller hippocampal volumes than those who were never depressed ($p < .05$), whereas participants with late-onset depression had smaller entorhinal cortex volumes ($p < .05$). HPA axis activity did not explain these differences.

Conclusions: We found differential associations of age of onset of depression on hippocampal and entorhinal cortex volumes, which could not be explained by alterations in HPA axis regulation.

Key Words: Cortisol, depression, entorhinal cortex, hippocampus, hypothalamic-pituitary-adrenal axis, neuroimaging

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 the disorder (1). Many studies observed smaller hippocampal volumes in patients with MDD compared with healthy control subjects (2). Recent studies suggest that particularly severe and recurrent depression are associated with hippocampal atrophy (1,2).

Moreover, some longitudinal studies have shown that a smaller hippocampal volume predicts a poorer clinical outcome (3,4). Furthermore, there is some evidence that depression-related hippocampal volume loss is reversible because it has been reported that patients with remitted depression show less hippocampal atrophy than patients with current depression (5). It is also thought that the age of onset of first depressive episode is of importance (6); an early onset of depression (EOD) is associated with recurrent depression and may therefore be associated with hippocampal atrophy (7). Thus far, inconsistent findings have been reported;

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smaller hippocampal volumes have been found in EOD (8) but also in late-onset depression (LOD) (9).

An explanation often proposed for the relation between MDD and smaller hippocampal volumes is dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis that may occur in MDD (10). The hippocampus plays an inhibitive role in regulating the HPA axis (11), and chronic exposure to glucocorticoids with repeated depressive episodes could lead to cell death and hippocampal atrophy (12,13). However, few studies have investigated whether HPA axis dysregulation mediates the relation between MDD and smaller hippocampal volumes, and one study among elderly depressed patients that did consider this did not find that cortisol levels mediated the association between depression and smaller hippocampal volumes (7).

The entorhinal cortex is also part of the medial temporal lobe and regulates memory function as well (14). 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 etiology of depression (15). Although the entorhinal cortex and hippocampus are closely related, the entorhinal cortex has rarely been investigated in relation to depression.

To our knowledge, no previous studies examined MDD and history of depressive episodes with hippocampal and entorhinal cortex volumes in a single population. Also, few studies have examined the role of HPA axis activity. The aim of this study was twofold. First, we investigated whether MDD, severity of depressive symptoms, and history of depressive episodes were associated with hippocampal and entorhinal cortex volumes. Second, we examined 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

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depression (16), HPA axis dysregulation (17), and brain atrophy (18) and may thus be more vulnerable for the potential detrimental effects of depression on hippocampal and entorhinal cortex volumes.

Methods and Materials

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 magnetic resonance imaging (MRI) in 1309 independently living participants with symptomatic atherosclerotic disease. Between 2001 and 2005, as part of the SMART-MR Study, an MRI investigation of the brain was added to the baseline examination in patients who were included with manifest coronary artery disease and had no MRI contraindications (pacemaker, claustrophobia, or pregnancy). Coronary artery disease was present in 59%, cerebrovascular disease in 23%, peripheral arterial disease in 22%, and abdominal aortic aneurysm in 9% of these patients. The cumulative percentage exceeds 100% because patients can have vascular disease at more than one location (19,20). Between 2006 and 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, medical history and a depression interview. This follow-up study is called the SMART-Medea (Memory, Depression, and Aging) study, which is aimed at investigating how brain changes are associated with psychosocial vulnerability and stress factors. 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.

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

Depression Measures

The presence of MDD (current MDD [cMDD]) in the preceding 12 months was assessed in all participants according to DSM-IV criteria using the Composites International Depression Interview (CIDI, version 2.1) (21).

History of depression was based on two core symptoms of the CIDI lifetime depression section, and the age of first depressive episode was assessed. The first episode of depression before age 50 years was classified as EOD, and the first episode at 50 years or older was classified as LOD (9).

On the basis of these assessments, we created three depression groups: never depressed; 12-month MDD (cMDD), and remitted MDD (participants with a history of depressive episodes, but no cMDD; remitted major depressive disorder [rMDD]). The cMDD and rMDD groups were combined into a group of those who ever had MDD ("ever-depression"). 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 previous 2 weeks was measured with the Patient Health Questionnaire (PHQ-9) (22,23), 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 0–27).

Brain Segmentation

The MR investigations were performed on a 1.5-Tesla whole-body system (Gyrosan ACSNT, Philips Medical Systems, Best, the Netherlands). See Supplement 1 for the MRI protocol.

Brain volumes were calculated with a probabilistic segmentation technique (18), and results of the segmentation analysis were visually checked for the presence of infarcts and adapted if necessary to make a distinction between white matter lesions (WML) and infarct volumes. Total brain volume was calculated by summing the volumes of gray and white matter and the volumes of WML and infarcts. Total intracranial volume (ICV) was calculated by summing the total brain volume and the volume of the cerebrospinal fluid.

Assessment of Hippocampal and Entorhinal Cortex Volume

The sagittal T1-weighted images were tilted to the coronal plane and oriented 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 (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 (Figure 1). Left and right entorhinal cortex and hippocampal volumes were calculated by multiplying the total number of voxels by the volume of a voxel ($1.0 \times .94 \times .94$ mm). See Supplement 1 for description of the manual segmentation protocol.

The intrarater reliability coefficient for repeated tracing in 20 randomly selected hippocampi was .96 and .98, and the interrater agreement between the two raters was .96. The coefficient of variation (24) for the two raters was 3.8%. The intrarater reliability

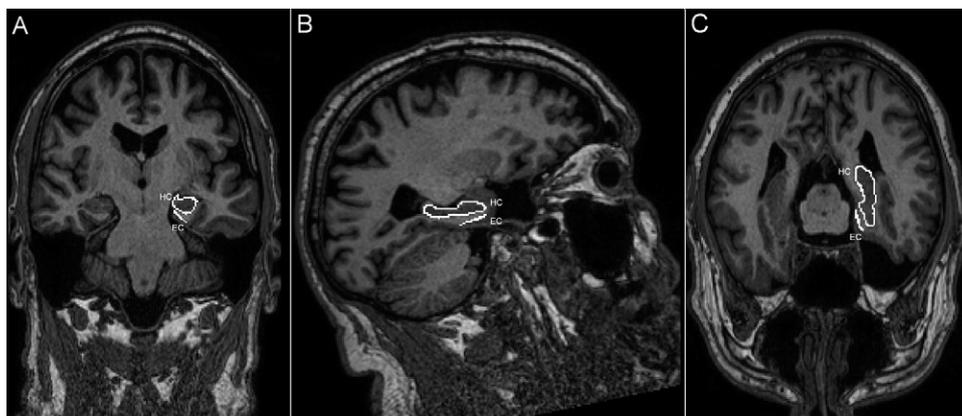


Figure 1. Magnetic resonance images of the hippocampal formation and entorhinal cortex. Shown are coronal (A), sagittal (B), and axial (C) images. EC, entorhinal cortex; HC, hippocampus.

coefficient for repeated tracing in 20 randomly selected entorhinal cortices was .92, and the coefficient of variation was 4.8%.

HPA Axis Activity

HPA axis activity was assessed at home by seven measurements of cortisol in saliva over a period of 24 hours to obtain the circadian rhythm (10). The saliva was collected using cotton dental rolls (Salivette, Sarstedt, Nümbrecht, Germany). Participants were instructed to refrain from smoking, drinking caffeine, eating, or brushing their teeth for at least 30 min before collecting saliva and to chew on the rolls for at least 2 min. On Day 1, participants were instructed to take the first sample on a regular weekday immediately after awakening while still lying in bed, and to take the second, third, and fourth samples after 30, 45, and 60 minutes, respectively. Samples 5 and 6 were collected at 10 PM and 11 PM, respectively. Furthermore, participants were asked to take .5 mg of dexamethasone orally after their sixth saliva sample, to sample their saliva the next morning directly after awakening, and had to record the time at which each saliva sample was taken. The cortisol in saliva was measured using an in-house competitive radioimmunoassay employing a polyclonal anticortisol-antibody (K7348). [1,2-3H(N)]-Hydrocortisone (NET185, NEN—DuPont, Dreiech, Germany) was used as a tracer. The lower limit of detection was .5 nmol/L and interassay 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 (25). Resting levels of cortisol were defined as the average of the saliva samples taken at 10 PM and 11 PM. As an indicator of suppression of the HPA axis, the cortisol value was taken at awakening the morning after the ingestion of the dexamethasone. Nonsuppression was defined as cortisol levels > 4.8 nmol/L. Because evening cortisol levels and awakening cortisol after the dexamethasone suppression test were skewed, these data were natural log-transformed.

Covariates

Educational level was divided into eight categories, graded from primary school to academic degree.

Height and weight were measured without shoes and heavy clothing, and the body mass index was calculated (kg/m²). 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 \geq 7.0 mmol/L, or self-reported use of oral antidiabetic drugs or insulin. Glucose was measured in an overnight fasting venous blood sample. 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 per week, 1–20 drinks per week, and > 20 drinks per week. Global cognitive functioning was assessed by the Mini-Mental State Examination (26).

Study Sample

Of the 754 participants who were examined between 2006 and 2009, a three-dimensional T1-weighted MRI of the brain was made in 649 participants, and 636 scans were without artifacts. 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 ($n = 432$).

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 data sets) to address the missing values (27,28) using the statistical program R (version 2.10.0). Data were analyzed using SPSS version 17.0 (Chicago, Illinois), by pooling the 10 imputed data sets. Volumes were divided by ICV and multiplied with the average ICV of the study population (1455 mL) to obtain relative volumes.

First, general linear models were created to estimate adjusted mean differences in hippocampal and entorhinal cortex volumes with ever-depression, irrespective of acuteness and age of onset, as independent variable. Second, general linear models were created to estimate adjusted mean differences in hippocampal and entorhinal cortex volumes, according to the three depression groups (cMDD, rMDD, never depressed). Third, 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). Fourth, with linear regression analysis the associations of depressive symptoms as measured with the PHQ-9 with hippocampal and entorhinal cortex volumes were estimated. All analyses were adjusted for age, sex, education, body mass index, diabetes mellitus, systolic and diastolic blood pressure, smoking habits, alcohol intake, antidepressant use and WML volumes. Additionally, to check whether results are specific to hippocampal and entorhinal cortex volumes, we divided them by total brain volume in separate analyses.

To investigate whether HPA axis activity explained or mediated a relation between depression and medial temporal lobe volumes, cortisol measures were added to the statistical models described earlier. Also, interaction terms between cortisol levels and depression measures were tested.

Results

Table 1 presents the characteristics of the study population according to depression status.

Overall, cMDD was diagnosed in 7.4% of the population, 37.6% reported a history of depressive episodes but were in remission (rMDD), and 55% had no history of depression. Within the group of ever-depressed subjects ($n = 286$), 72% had an EOD and 28% had a LOD. There was a significant difference between cMDD and rMDD with regard to age of onset; of the participants with cMDD, 53% had an early onset of depression, whereas 75% of the participants with rMDD had an early onset of depression ($\chi^2 = 5.42$; $p = .01$).

Compared with participants who had never had depression, ever-depression, irrespective of acuteness and age of onset, was associated with smaller left-sided hippocampal volumes (left: $B = -.053$; 95% confidence interval [CI]: .011–.00; $p = .05$) but not significantly on right-sided volumes ($B = -.048$; 95% CI .010–.002; $p = .12$). With regard to entorhinal cortex volume, there was a trend for smaller right-sided volumes in persons with ever-depression ($B = -.007$; 95% CI .015–.001; $p = .07$) but not for left-sided volumes ($B = -.005$; 95% CI .012–.002; $p = .15$).

Participants with cMDD did not have significantly smaller hippocampal volumes (mean difference: $-.07$ mL; 95% CI $-.18$ to $.04$ mL; $p = .21$) or entorhinal cortex volumes (mean difference: $-.001$ mL; 95% CI $-.011$ to $.009$ mL; $p = .84$) than those who were never depressed (Figure 2A and 2B).

Also, depressive symptoms were not associated with hippocampal volumes (left: B per point increase on PHQ-9 $-.001$ mL, 95% CI $-.01$ to $.01$, $p = .89$; right: B .003 mL; 95% CI $-.01$ to $.01$; $p = .53$),

Table 1. Baseline Characteristics According to Depression Group

	Never (<i>n</i> = 350)	rMDD (<i>n</i> = 239)	cMDD (<i>n</i> = 47)	EOD (<i>n</i> = 200)	LOD (<i>n</i> = 86)
Demographics					
Age	62 (9)	62 (9)	58 (10)	60 (10)	65 (9)
Male %	82	81	76	75	90
Level of education (0–8)	4 (2)	4 (2)	4 (2)	4 (2)	4 (2)
Vascular Risk Factors					
Body mass index	28 (4)	27 (4)	28 (4)	27 (4)	27 (5)
Systolic blood pressure (mm Hg)	144 (19)	142 (19)	143 (20)	141 (17)	144 (21)
Diastolic blood pressure (mm Hg)	82 (11)	82 (11)	84 (11)	83 (11)	80 (11)
Diabetes mellitus %	23	19	16	19	24
Smoking (pack-years) ^a	16 (0–38)	23 (0–52)	21 (0–49)	24 (0–53)	15 (0–49)
Alcohol use %					
< 1 drinks per week	31	30	36	14	12
1–20 drinks per week	58	59	55	75	78
> 20 drinks per week	11	11	9	11	10
Mini-Mental State Examination	29 (27–30)	29 (27–30)	29 (27–30)	29 (27–30)	29 (27–30)
Brain Measures					
Intracranial volume (mL)	1454 (126)	1465 (106)	1442 (147)	1463 (124)	1464 (143)
Total brain volume (mL)	1139 (105)	1145 (106)	1137 (119)	1148 (108)	1128 (113)
White matter lesion volume (mL) ^a	1.3 (.3–9.6)	1.3 (.3–6.5)	1.4 (.4–7.0)	1.3 (.4–7.9)	1.6 (.5–9.0)
Crude hippocampal volume (mL)					
Left	2.97 (.36)	2.94 (.38)	2.90 (.32)	2.94 (.37)	2.93 (.38)
Right	3.00 (.39)	3.00 (.43)	2.91 (.35)	2.99 (.43)	2.94 (.38)
Crude entorhinal cortex volume (mL) ^b					
Left	.17 (.033)	.17 (.034)	.17 (.035)	.17 (.035)	.16 (.034)
Right	.17 (.034)	.17 (.034)	.17 (.036)	.17 (.034)	.16 (.036)
Depression Characteristics					
Depressive symptoms ^{a,c}	1.0 (.0–5.0)	2.0 (.0–8.0)	7.0 (2.2–17.0)	3.0 (.0–9.0)	3.0 (.0–9.0)
Age of onset of depression	NA	40 (14)	43 (17)	35 (11)	56 (6)
Use of antidepressants %	4	8	31	13	11
Early onset %	NA	74	64	100	0
Late onset %	NA	26	36	0	100
HPA Axis Activity					
AUCg Morning Cortisol nmol/L per Hour	17.4 (7.1)	17.7 (8.2)	17.0 (6.8)	16.8 (6.7)	18.2 (7.3)
Evening Cortisol nmol/L ^a	3.4 (2.0–6.2)	3.1 (1.8–7.9)	3.8 (2.3–7.5)	3.2 (2.0–6.1)	3.7 (2.2–6.2)
Awakening Cortisol after DST nmol/L ^a	1.6 (.9–3.5)	1.9 (.7–3.1)	1.8 (.6–3.0)	1.6 (.7–3.2)	1.7 (.8–4.0)
—nonsuppression (%) ^d	7	5	6	5	6

AUCg, area under the curve to the ground; cMDD, current MDD; DST, dexamethasone suppression test; EOD, early onset depression; HPA, hypothalamic pituitary adrenal; LOD, late onset depression; MDD, major depressive disorder; rMDD, remitted MDD.

Data are presented as means with standard deviations unless otherwise specified.

^aData are presented as medians with 10% to 90% intervals.

^bAssessed in 479 participants.

^cMeasured with the Patient Health Questionnaire—9.

^dDST nonsuppression was determined as cortisol L > 4.8 nmol/L.

or with entorhinal cortex volumes (left: $B = .001$ mL, 95% CI $-.001$ to $.000$; $p = .21$; right: $B = .000$ mL; 95% CI $-.001$ to $.001$; $p = .45$). When depressive symptoms were added to the analyses between depression groups and brain volumes, none of the associations changed.

Participants with rMDD had smaller entorhinal cortex volumes than those who were never depressed (mean difference: $-.008$ mL; 95% CI $-.015$ to $-.001$ mL; $p = .02$), but no significant difference was found on hippocampal volumes (mean difference: $-.05$ mL; 95% CI $-.10$ to $.01$ mL; $p = .10$; Figure 2A and 2B).

We further explored the role of age of onset of depression by differentiating EOD and LOD within participants who were remitted or had MDD. Participants with EOD had smaller hippocampal volumes than those who were never depressed (mean difference: $-.06$ mL; 95% CI $-.123$ to $-.001$ mL; $p = .04$) but did not have smaller entorhinal cortex volumes (Figure 3A and 3B). Participants with LOD had smaller entorhinal cortex volumes than participants never depressed (mean difference: $-.009$ mL; 95% CI $-.017$ to

$-.001$ mL; $p = .03$), whereas no significant differences in entorhinal cortex volumes were found for EOD versus never depressed (Figure 3A and 3B). Among subjects with current MDD ($n = 47$), we found no effect of age of onset on hippocampal or entorhinal cortex volume (results not shown; $p > .05$).

The percentual differences in hippocampal volume ranged from -2.8% in cMDD to -0.3% in LOD, whereas the percentual differences on entorhinal cortex volume ranged from -6.1% in LOD to $+2.1\%$ in cMDD (Table 2). To examine whether these findings were selective for the hippocampus and entorhinal cortex, we repeated all analyses in which hippocampus and entorhinal cortex volumes were divided by total brain volume, and this resulted in similar significant findings.

To examine whether the effect of depression group (cMDD, rMDD, and never MDD) is affected by age of onset, we added an interaction term between depression group and age of onset of depression to the fully adjusted model but found no significant interactions ($p > .05$).

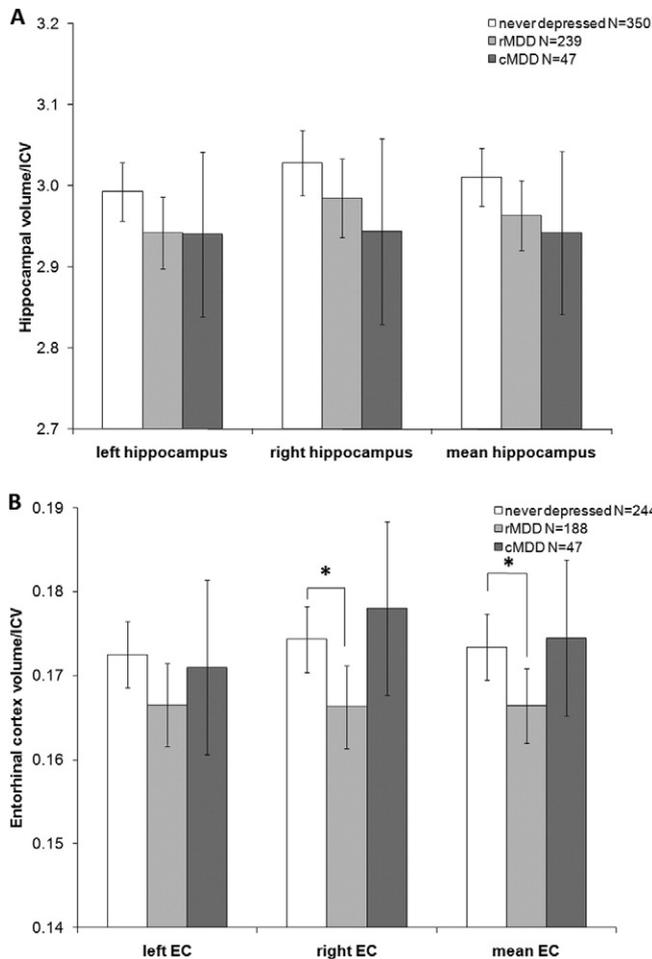


Figure 2. Adjusted means of (A) hippocampal and (B) entorhinal cortex (EC) volumes, divided by intracranial volume (ICV) according to depression groups error bars represent 95% confidence intervals; * $p < .05$. Mean volumes (in milliliters) are adjusted for age, sex, level of education, blood pressure, diabetes mellitus, body mass index, pack-years of smoking, alcohol consumption, antidepressant use, and volume of white matter lesions. cMDD, current major depressive disorder; rMDD, remitted major depressive disorder.

Ever-depression and depressive symptoms were not associated with significant differences in cortisol levels ($p > .05$; data not shown). The three depression groups (never, rMDD, cMDD) did not differ in levels of cortisol (Figure 4A). However, compared with subjects who were never depressed, LOD was associated with higher area under the curve to the ground (mean difference: 2.01; 95% CI .42–3.60; $p = .013$), whereas EOD was not ($p = .80$; Figure 4B). LOD and EOD were not associated with evening cortisol levels and awakening cortisol after dexamethasone suppression test nor was there a difference in the number of nonsuppressors between the depression groups ($p > .05$).

When we added cortisol measures to the models, none of the associations were altered, suggesting that HPA axis activity did not explain or mediate the relation of depression measures with brain volumes. Also, we found no evidence for cortisol-mediated hippocampal or entorhinal cortex volume loss in depression because none of the interaction terms between depression and cortisol measures were statistically significant (p value for interaction terms $> .05$).

We reanalyzed our data excluding patients with a history of cerebrovascular disease (including (non)ischemic stroke and tran-

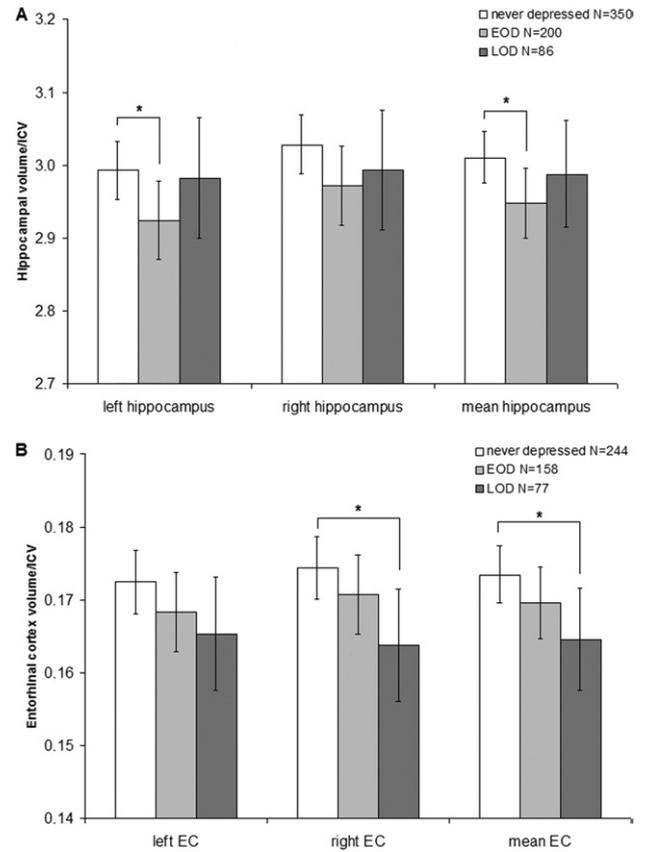


Figure 3. Adjusted means of (A) hippocampal and (B) entorhinal cortex (EC) volumes, divided by intracranial volume (ICV), according to age of onset of first depressive episode. Error bars represent 95% confidence intervals; * $p < .05$. Mean volumes (in milliliters) are adjusted for age, sex, level of education, blood pressure, diabetes mellitus, body mass index, pack-years of smoking, alcohol consumption, antidepressant use, and volume of white matter lesions. EOD, early-onset depression; LOD, late-onset depression.

sient ischemic attack; $n = 162$), and none of the associations changed.

Discussion

In this large cohort study of participants with a history of atherosclerotic disease, ever-depression, irrespective of acuteness and

Table 2. Percentual Differences in Hippocampal and Entorhinal Cortex Volumes per Depression Group

	Ever MDD	cMDD	rMDD	EOD	LOD
Hippocampal Volume					
Mean	-1.7% ^a	-2.3%	-1.6%	-2.1% ^b	-.8%
Right	-1.6%	-2.8%	-1.4%	-1.9%	-1.2%
Left	-1.7% ^b	-1.7%	-1.7%	-2.3% ^b	-.3%
Entorhinal Cortex Volume					
Mean	-3.5% ^a	+ .6%	-4.0% ^b	-2.3%	-5.1% ^b
Right	-4.0% ^a	+2.1%	-4.6% ^b	-2.1%	-6.1% ^b
Left	-2.8%	-.9%	-3.5%	-4.2%	

cMDD, current major depressive disorder; EOD, early-onset depression; LOD, late-onset depression; rMDD, remitted major depressive disorder.

^aTrend for significant difference, compared with never depression ($.05 > p < .07$).

^bSignificantly different from never depression ($p < .05$).

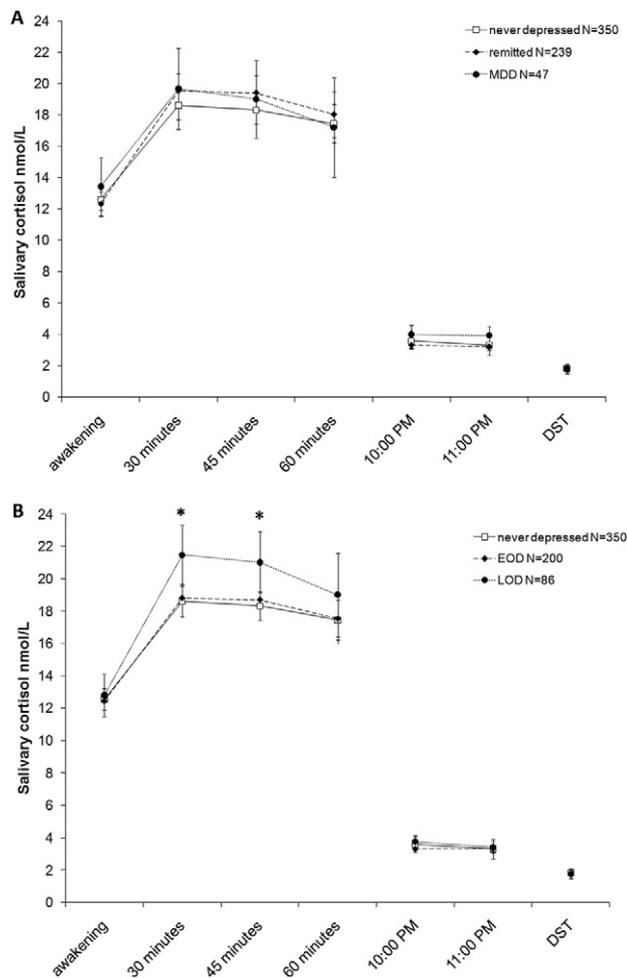


Figure 4. Adjusted salivary cortisol levels according to (A) depression groups and (B) age of onset of depression. Error bars represent 95% confidence intervals; * $p < .05$. Mean levels are adjusted for age, sex, educational level, systolic and diastolic blood pressure, diabetes mellitus, body mass index, pack-years of smoking, alcohol consumption, antidepressant use, and time of awakening. MDD, major depressive disorder; EOD, early-onset depression; LOD, late-onset depression.

age of onset, was associated with smaller hippocampal volumes but not significantly with entorhinal cortex volumes. In addition, participants with depression in remission had smaller entorhinal cortex volumes and a differential association was observed for EOD and LOD; LOD was associated with smaller entorhinal cortex volumes, whereas EOD was associated with smaller hippocampal volumes. HPA axis activity did not explain any of 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 (29), and some also found associations with smaller entorhinal cortex volumes (16,30). Within these studies, the role of HPA axis was, however, not investigated.

Thus far, the relation between a history of depression and entorhinal cortex volumes has not been investigated. We found a 5.1% reduction in entorhinal cortex volume in participants with LOD, whereas this difference was only .8% in hippocampal volume, and in participants with EOD, the reduction was 2.3% and 2.1% in ento-

rhinal cortex and hippocampal volume, respectively. Compared with the effect of aging, the effect of LOD on entorhinal cortex volume was stronger, whereas the effect of EOD on hippocampal volume and entorhinal cortex was only half of the effect of aging.

It has previously been found that hippocampal volumes are smaller in patients with depression than in patients in remission (3). We also found that patients with ever-depression had smaller hippocampal volumes than never depressed subjects. Additionally, we found that subjects with rMDD had significantly smaller entorhinal cortex volumes than those with MDD or those who were never depressed. However, this is most likely explained by the association with LOD, because most participants with LOD were in remission.

Our finding that EOD, but not LOD, was associated with smaller hippocampal volumes, is in line with results from one preceding study (31), whereas other studies found more hippocampal volume loss in LOD than in EOD (9,32). It has frequently been suggested that EOD is associated with recurrent depression and that hippocampal volume loss could be a consequence of chronic or repetitive exposure to stress-related neurotoxic factors (33).

Recently, we reported data from this cohort showing that higher evening levels of cortisol and reduced suppression after dexamethasone were associated with smaller hippocampal volumes (13). In the present study, we show that HPA axis regulation does not explain the observed relation between EOD and smaller hippocampal volumes because EOD was not associated with alterations in basal HPA axis regulation. In contrast to EOD, we observed that LOD was associated with higher basal morning cortisol levels. Elevated levels of cortisol have previously been found in depressed elderly (34), and it is thought that elevated levels of cortisol can result in cognitive decline and brain atrophy (35). However, also for LOD, we found no evidence for glucocorticoid-mediated hippocampal and entorhinal cortex volume loss. To our knowledge, only two previous studies examined whether cortisol levels could explain the smaller hippocampal volumes in depression, and these studies also did not find any proof for glucocorticoid-mediated hippocampal volume loss in depression (7,36).

An alternative explanation for our findings is that smaller hippocampal volumes antedates the onset of depression early in life, as has also been suggested in the onset of posttraumatic stress disorder (37). Two studies that reported smaller hippocampal volumes in nondepressed subjects with a familial risk for depression suggest that smaller hippocampal volumes constitute a vulnerability to develop depression (38,39). Also, it has recently been shown that subjects with a history of childhood abuse are particularly at risk for smaller hippocampal volumes if they have a genetic predisposition (40,41). Further studies are needed to investigate whether hippocampal volume loss is a cause or a consequence of depression.

Whereas EOD is thought to be a result of the interplay between genetic predisposition and stressful experiences, LOD is thought to be a result of vascular brain pathology (6). LOD has frequently been associated with WML (42). Within our population, we found no significant crude association between depression groups and WML volume. Also after adjustment for WML our associations did not change. Therefore, it seems unlikely that the relation between LOD and smaller entorhinal cortex volume can be explained by WML. One possible explanation for these findings is that LOD occurred because loss of entorhinal cortex volume represents a prodromal phase of Alzheimer's disease. A differential association of hippocampal and entorhinal cortex volumes has been observed in relation to Alzheimer's disease in which it is thought that the entorhinal cortex is affected in an earlier stage of the disease than the hippocampus (43). Thus, it is possible that even though our partic-

ipants were relatively young, LOD represents a prodromal phase of Alzheimer's disease in our study (44).

Our study has several strong aspects, the first of which is its large sample size. Furthermore, we were able to investigate whether HPA axis activity 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 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. We had data on important covariates and precise measurements of brain volumes, which made it possible to adjust associations for ICV, total brain, and WML.

A limitation of our study is the cross-sectional design, and therefore cause and consequence cannot be discerned. HPA axis activity was measured on one day, whereas multiple days of sampling are necessary to measure salivary cortisol levels reliably, particularly directly after awakening (45). This was not an option in our large-scale study, however, and we believe that the reliability of individual measures is likely to be compensated by the large sample size of our study. By assessing history of depression, we tried to investigate retrospectively 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 resulting in an overestimation of depression in the past. However, it is also possible that more severe depressive episodes were more often recalled. Furthermore, we had no data on duration of illness and number of depressive episodes. Finally, the entorhinal cortex was outlined in a random subset of subjects in which the hippocampus was already outlined. This may have resulted in loss of statistical power to find significant associations. Our study population consisted of patients with a history of arterial disease. Because several vascular risk factors have been associated with depression, altered basal HPA axis activity, and brain atrophy, we do not know to what extent the observed associations can be generalized to the general population.

To summarize, in this cohort, ever-depression was associated with smaller hippocampal volumes, but not with entorhinal cortex volumes. Late-onset depression and remitted depression were associated with smaller entorhinal cortex volumes, whereas early-onset depression was associated with smaller hippocampal volumes. We found no evidence for glucocorticoid-mediated hippocampal or entorhinal cortex volume loss. More studies in single populations, preferably prospective, are needed to unravel further the relation among depression, HPA axis activity, and brain volumes.

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Supplementary material cited in this article is available online.

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