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Cortisol relates to regional limbic system structure in older but not younger adults

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ABSTRACT

We investigated if the relationship between age and regional limbic system brain structure would be moderated by diurnal cortisol output and diurnal cortisol slope. Participants aged 23–83 years collected seven salivary cortisol samples each day for 10 consecutive days and underwent magnetic resonance imaging. Age, sex, cortisol, and an age x cortisol interaction were tested as predictors of hippocampal and amygdalar volume and caudal and rostral anterior cingulate cortex (ACC) thickness. We found significant interactions between age and cortisol on left and right amygdalar volumes and right caudal ACC thickness. Older adults with higher cortisol output had smaller left and right amygdalar volumes than older adults with lower cortisol output and younger adults with higher cortisol output. Older and younger adults with lower cortisol output had similar amygdalar volumes. Older adults with a steeper decline in diurnal cortisol had a thicker right caudal ACC than younger adults with a similarly shaped cortisol slope. Hippocampal volume was not related to either cortisol slope or output, nor was pallidum volume which was assessed as an extra-limbic control region. Results suggest that subtle differences in cortisol output are related to differences in limbic system structure in older but not younger adults.

1. Introduction

The hippocampus, amygdala, and anterior cingulate cortex (ACC), regions within the limbic system, are targets of glucocorticoid (GC) actions due to their rich expression of glucocorticoid receptors (GRs) (Morimoto et al., 1996; Wang et al., 2013, 2014). Although short-term GC elevations can result in adaptive neural plasticity, such as long-term potentiation (LTP), chronic elevations of circulating GCs can promote maladaptive change, such as disruption of LTP (Sapolsky, 2003). Animal studies indicate that chronic exposure to elevated GCs or chronic stress can engender dendritic atrophy within the hippocampus (Magariños and McEwen, 1995), ACC (Radley et al., 2004), and medial amygdala (Bennur et al., 2007), but dendritic arborization in the basolateral amygdala (Vyas et al., 2002). In humans, GC therapy or grossly elevated endogenous GCs due to Cushing's syndrome are related to smaller hippocampal and amygdalar volumes in younger and middle-aged adults (Brown et al., 2004, 2008; Merke et al., 2003; Starkman et al., 1992). Gray matter volume in the ACC is also smaller in adult patients with long-term remission of Cushing's disease relative to controls (Andela et al., 2013).

These studies suggest that highly elevated circulating cortisol, the primary GC in humans, has deleterious effects upon limbic system structure. However, the influence of more subtle increases of cortisol is far less clear, with studies reporting negative (Lupien et al., 1998; Wolf et al., 2002) and statistically non-significant (Kremen et al., 2010; MacLullich et al., 2005) associations between cortisol and hippocampal volume. Few studies have examined the relationship of non-pathological increases in cortisol to ACC structure (Kremen et al., 2010; Wolf et al., 2002), and no study to our knowledge has described the association of endogenous cortisol to amygdalar volume in healthy adults. Furthermore, most human studies investigating cortisol and limbic system structure have focused on older or middle-aged adults (Kremen et al., 2010; Lupien et al., 1998; MacLullich et al., 2005). Thus, it is not known if the relationship of age to limbic system structure is exacerbated by increased cortisol exposure.

Results from one rodent study suggest that moderate, sustained

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stress over a six-month period promotes neurodegeneration in the hippocampus of older but not younger adult animals (Kerr et al., 1991). The stress manipulation reduced hippocampal pyramidal cell density in older rats relative to non-stressed same-aged controls, but had little effect on pyramidal cell density in younger adult rodents relative to same-aged non-stressed controls. Unlike the moderate stress protocol in that study, high stress paradigms or the administration of high doses of GCs do cause dendritic atrophy in young adult rodents (Sapolsky, 2003). It is possible that younger neural tissue is only sensitive to higher levels of GCs, whereas older neural structure is sensitive to moderate stress and lower levels of GCs. Lupien et al. (1998) reported smaller hippocampi in older adults with increasing and currently-high cortisol levels relative to older adults with decreasing and currently-moderate cortisol levels, but their study did not investigate younger adults, and thus did not allow comparison between the two age groups.

Studies examining the influence of age and stress or GC exposure on the ACC are lacking. Past studies in rodents have focused on young adult animals alone (e.g., Radley et al., 2004) and some studies in humans have focused on one adult age group (Kremen et al., 2010). In a study of younger and older adult humans, elevated cortisol was related to smaller hippocampi, but not total (anterior plus posterior) cingulate gyrus volume; however, increased morning adrenocorticotropic hormone (ACTH) was associated with both smaller hippocampi and total cingulate gyrus volume (Wolf et al., 2002). The interaction of age and cortisol or ACTH on hippocampal or total cingulate gyrus volumes was not examined.

The effect of short-term stress on amygdalar structure was compared in both younger and older adult rodents (Marcuzzo et al., 2007). One hour of restraint stress decreased dendritic spine density in the posterior dorsomedial amygdala in both age groups (Marcuzzo et al., 2007). Exposure to extended GC therapy for treatment of asthma or rheumatic diseases has been shown to shrink the adult human amygdala (Brown et al., 2008), and financial hardship, which presumably would be associated with elevated cortisol, has been related to smaller amygdalar volumes in middle-aged adults (Butterworth et al., 2012). More research is needed to clarify the relationship of cortisol increase to the structure of the human amygdala and to examine whether cortisol moderates the relationship of age on this limbic region.

To obtain a reliable estimate of an individual's cortisol profile, multiple days of collection are required (Segerstrom et al., 2014). Four- to 8-days of sampling have been recommended for area under the curve and 10 days for cortisol slope (Segerstrom et al., 2014). The lack of consistency between studies relating cortisol to hippocampal volume could be due to limited cortisol sampling which results in substantial measurement error when assessing chronic levels of the hormone. Most past studies have collected cortisol over 1 day instead of several days (Kremen et al., 2010; MacLullich et al., 2005; Wolf et al., 2002). Measures from a single day generally reflect influences from situational or state-like factors and not more stable trait-like characteristics (Ross et al., 2014). To achieve adequate measurement reliability and hence measures representative of chronic cortisol output and dynamics, we collected cortisol seven times/day for 10 consecutive days.

In the present study, we recruited a lifespan sample of healthy adults (aged 23–83 years) who provided salivary cortisol samples seven times/day, from morning to evening over 10 consecutive days, and who later underwent magnetic resonance imaging (MRI). We investigated the following: 1) the relationship of cortisol to hippocampal and amygdalar volume and ACC thickness, and 2) cortisol as a moderator of the relationship of age on hippocampal and amygdalar volume and ACC thickness. We hypothesized that older adults with less healthy cortisol measures (i.e., increased cortisol output or less negative cortisol slope) had smaller hippocampal and amygdalar volumes and thinner ACC than older adults with more healthy cortisol measures and younger adults, regardless of their cortisol level.

2. Method

2.1. Participants

Participants were initially recruited from the Atlanta, GA metropolitan area to participate in a study investigating everyday problem solving and emotion regulation. Individuals were excluded if they reported any of the following conditions known to influence HPA axis function: 1) pregnancy, 2) post-traumatic stress disorder, 3) bipolar disorder, 4) psychosis, 5) eating disorder, 6) dementia, 7) Cushing's disease, or 8) Addison's disease (see Nater et al., 2013). People were also excluded if they reported a recent major stressful life event (e.g., death in the family or surgery), indicated use of recreational drugs, had a history of alcohol abuse, or had a schedule (e.g., shift work) that would interfere with cortisol collection. The initial study included 185 participants. Sixty-four of these agreed to return to participate in a further MRI investigation, which occurred 8 to 38 months (M = 28.33; SD = 7.73) later. Seven were excluded due to MRI contraindications. One participant was excluded due to lack of cortisol data (i.e., only three out of 70 assessments were collected). Four persons with neurological abnormalities (i.e., right medial temporal lobectomy, space-occupying lesion in right temporal lobe, frontal lobe lesion, and right hemisphere arachnoid cyst) were excluded. Out of the remaining 52 participants, one with right amygdalar volume less than 3 *SDs* from the sample mean was also excluded. The final sample (N = 51) ranged in age from 23 to 83 years (M = 53.10, SD = 18.04; female = 24) and contained similar numbers of younger (23 to 43 years; N = 17), middle-aged (46 to 62 years; N = 15), and older (62 to 83 years; N = 19) adults. The participants in the final sample did not significantly differ in age, $t(183) = -1.17$, $p = .24$, or cortisol area under the curve, $t(182) = 0.43$, $p = .66$, from the other participants in the initially recruited sample. Individuals provided informed consent prior to participating in the initial and follow-up study.

2.2. Procedure

Participants provided seven daily saliva samples for 10 consecutive days using the Salivette® saliva collection system (Sarstedt AG & Co., Nümbrecht, Germany). Saliva was collected upon waking, 30 min following waking, and every 3 h until the evening. A Tungsten T handheld computer (Palm, Inc.) beeped at specific times to remind participants to collect samples. Participants documented collection times in the handheld device.

Cortisol (nmol/L) was analyzed from saliva using a commercial chemiluminescence immunoassay (IBL, Hamburg, Germany; see Nater et al., 2013 for collection details). Assays were conducted in Professor Kirschbaum's laboratory at the Technical University of Dresden. To address cortisol outliers, we calculated a within-person cortisol mean for each individual and removed any cortisol value deviating more than 3 *SDs* from the within-person mean. Out of a maximum of 3570 possible cortisol values (seven samples per day x 10 days x 51 participants), we obtained a total of 3336 values meeting criteria for further analysis (93%).

2.3. Measures

2.3.1. Diurnal cortisol output

To measure diurnal cortisol output, area under the curve with respect to ground (AUC) was calculated using the trapezoid formula (Pruessner et al., 2003). AUC was not calculated for days when the awakening specimen and/or greater than three specimens were missing. A large majority of participants (72.5%) had sufficient cortisol data to calculate AUC for all 10 days. There were no participants who had less than 6 days of AUC data.

Table 1
Descriptive statistics of study variables (N = 51).

	N (%)	Mean (SD)	Range
Age (years)		53.10 (18.04)	23–83
Sex (female)	24 (47.1)		
Race (white)	39 (76.5)		
Education (years)		15.92 (1.74)	12–20
Smoker (yes)	6 (11.8)		
BMI		26.90 (5.20)	17.23–42.41
Diabetes (yes)	6 (11.8)		
Hypertension (yes)	14 (27.5)		
CES-D		8.43 (8.30)	0–39
Time between cortisol and MRI (mos.)		28.33 (7.73)	8–38
Cortisol output (AUC)		99.21 (25.55)	37.53–163.62
Linear cortisol slope		−.85 (.25)	−1.26 to −.37
Quadratic cortisol slope		.06 (.03)	−.05 to .13
L Hippocampal volume[a]		4,156 (494)	3,266–5,228
R Hippocampal volume[a]		4,293 (479)	3,021–5,265
L Amygdalar volume[a]		1,615 (246)	1,161–2094
R Amygdalar volume[a]		1,609 (239)	1,003–2,055
L Rostral ACC thickness		2.75 (.19)	2.35–3.24
R Rostral ACC thickness		2.62 (.21)	2.23–3.27
L Caudal ACC thickness		2.67 (.23)	1.95–3.15
R Caudal ACC thickness		2.46 (.21)	2.10–3.06

Note. ACC: Anterior cingulate cortex; BMI: Body mass index; CES-D: Center for Epidemiologic Studies Depression Scale; L: Left; R: Right; thickness = mm; volume = mm^3.

[a] All regional brain volumes were adjusted for intracranial volume.

2.3.2. Diurnal cortisol slope

Linear and quadratic cortisol slopes were estimated using a linear mixed model. Each cortisol sample across the day was used to calculate slopes, except for the 30 min post awakening sample, which was excluded to prevent the cortisol awakening response from obscuring the slope calculation. The term for measurement occasion (i.e., time) was centered around each individual's mean to reduce nonessential collinearity. Linear and quadratic terms of measurement occasion were entered as Level 1 predictors of cortisol. We calculated slopes for each day of data. Convergence criteria were met for 9 out of 10 days of data. Larger negative linear slope values represented greater decline in cortisol and larger positive quadratic slope values indicated greater decline in cortisol across the morning and a slightly greater increase in cortisol in the evening. A larger negative linear cortisol slope was a slope with a more negative value. We interpreted a more negative cortisol slope as indicative of a healthy diurnal cortisol rhythm.

We calculated within-person means for the cortisol measures (i.e., diurnal cortisol output and slope) by averaging measures across collection days for each person. The cortisol means reported in Table 1 represent the average of all the within-person means in the sample.

2.3.3. MRI acquisition and processing

Anatomical MRI images were acquired on a Siemens Magnetom Tim Trio 3-Tesla scanner at the Georgia State / Georgia Tech Center for Advanced Brain Imaging in Atlanta, GA. A sagittal T1-weighted MPRAGE sequence was used (echo time = 3.98 ms, repetition time = 2250 ms, inversion time = 850 ms, flip angle = 9 degrees, acquisition matrix = 256 × 265 × 176, and voxel size 1.0 × 1.0 × 1.0 mm). Freesurfer 5.3 (http://surfer.nmr.mgh.harvard.edu) was used to reconstruct, segment, and parcellate the brain in 86 gray matter regions (68 cortical, 16 subcortical and two cerebellar regions) (Desikan et al., 2006; Fischl et al., 2002). Careful visual inspection of the gray-white matter and gray matter-pial boundaries was conducted by highly trained operators who manually corrected errors, according to FreeSurfer specifications, until surface boundaries did not present anomalies. These corrections were done blind with respect to age and cortisol status. Selected limbic system regions are displayed in Fig. 1.

We included hippocampus and amygdala volume and rostral and caudal ACC thickness in our analyses because these specific regions of interest (ROIs) are known to be rich in glucocorticoid receptors. We focused on ACC thickness rather than surface area because past research has suggested that cortical thickness, and not surface area, is related to cortisol (Kremen et al., 2010). We chose also not to examine ACC volume because the volume of a cortical region is a product of its surface area and thickness. Each hemisphere was tested independently based on literature linking steroid exposure and steroid receptor distributions to cerebral asymmetry (Diamond, 1991).

To demonstrate the specificity of the association of cortisol to limbic system regions rich in GRs, we selected the pallidum as a control region due to its less dense population of GRs (Morimoto et al., 1996). Intracranial vault volume was manually traced by experts to adjust

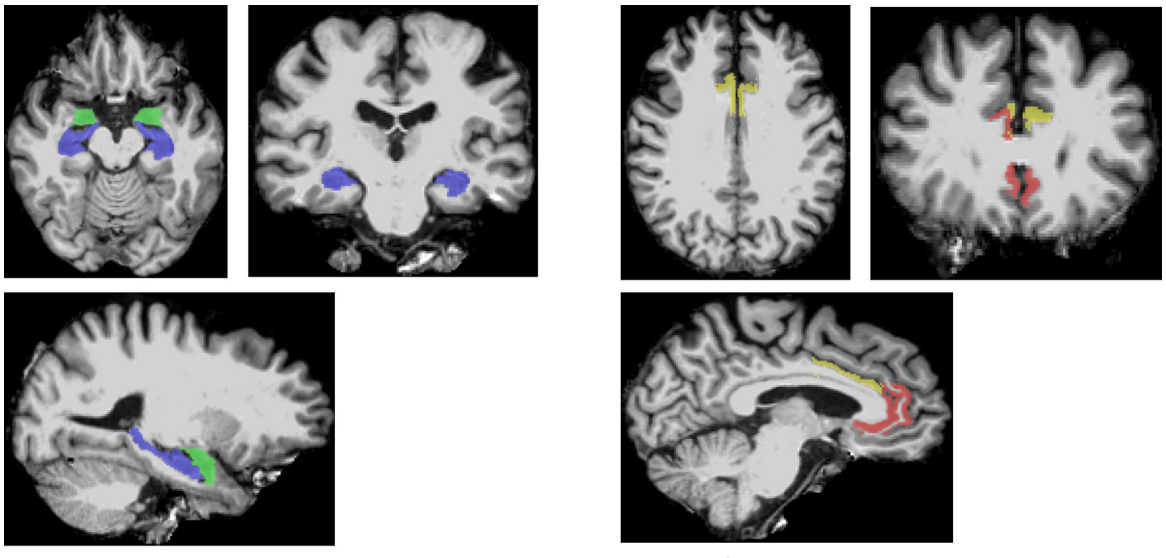

Fig. 1. Representative views of limbic system segmentations from Freesurfer 5.3 following manual correction. Fig. 1**a.** Axial, coronal and sagittal sections showing hippocampal (blue) and amygdala (green) volume segmentations. Fig. 1**b.** Axial, coronal and sagittal sections showing rostral (red) and caudal (yellow) anterior cingulate gyrus thickness parcellations (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

individual regional brain volumes for differences in head/body size. Hippocampal, amygdalar, and control volumes were residualized for intracranial volume (ICV) where: $Vol_{adj} = Vol - b(ICV - mean\ ICV)$ (Hansen et al., 2015). Vol_{adj} was the ICV-corrected regional brain volume, *Vol* was the original uncorrected brain volume, and *b* was the slope from the linear regression of *Vol* on *ICV* (Raz et al., 2004). ACC thickness, as convention, was not adjusted for ICV.

2.4. Statistical approach

Zero-order correlations were performed to test the relationship between age, sex, cortisol, and regional limbic system structure. Hierarchical linear regression was used to test if cortisol moderated the relationship between age and regional limbic system structure. Age at time of MRI was used in all analyses. Age and sex were entered into a first step (Model 1), cortisol was entered in the second step (Model 2), and the Age X Cortisol interaction term was entered in the third step (Model 3). Age and cortisol measures were mean-centered to reduce multicollinearity effects. Diurnal cortisol output and diurnal cortisol slope were tested in separate hierarchical linear regression models. We used hierarchical linear regression to test whether the variance in limbic system structure explained by the Age X Cortisol interaction was significantly greater than the overall variance explained by age, sex, and cortisol. When Age X Cortisol was a significant predictor, a secondary analysis was conducted using linear regression with the following additional covariates entered: time interval between cortisol collection and MRI, body mass index (BMI), current cigarette smoker, self-report of hypertension, self-report of diabetes, and depression symptoms measured using the Center for Epidemiologic Studies Depression Scale (CES-D; Radloff, 1977). Health-related factors that change with age were entered as covariates because they may account for significant Age X Cortisol interactions. We utilized a *p* value of .05 to indicate statistical significance for all tests; corrections for the number of tests conducted were not made. Using G*Power v. 3.1.6 (Faul et al., 2009) to calculate statistical power, we determined that we had 77% power to detect a significant ($p = .05$) interaction, with a medium effect size ($f^2 = 0.15$), in a linear multiple regression model with 4 (age, sex, cortisol output, and age x cortisol output interaction) or 5 (age, sex, linear cortisol slope, quadratic cortisol slope, and age x cortisol slope interaction) predictors.

3. Results

3.1. Participant characteristics

Descriptive statistics of the study sample can be found in Table 1. Age did not significantly correlate with sex, race, or education, indicating that these demographic characteristics did not vary with age. Correlations between primary study variables can be found in Table 2.

3.2. Hippocampal volume

Model 1 containing age and sex accounted for 42.7% and 46.8% of the variance in left and right hippocampal volume, respectively. Older adults had significantly smaller hippocampi than younger adults (left: $B = -17.60$, $\beta = -0.64$, $p = < .001$, $r = -0.65$; right: $B = -17.83$, $\beta = -0.67$, $p = < .001$, $r = -0.68$) and there was a trend for men to have a larger right hippocampus than women (left: $B = 166.19$, $\beta = 0.34$, $p = .13$, $r = 0.22$; right: $B = 177.56$, $\beta = 0.37$, $p = .08$, $r = 0.25$). The addition of cortisol in Model 2 did not explain any additional variance in hippocampal volume. Neither cortisol output nor slope (linear or quadratic) moderated the relationship of age on left or right hippocampal volume in Model 3.

3.3. Amygdalar volume

Model 1 containing age and sex accounted for 28.8% and 24.9% of the variance in the size of the left and right amygdala, respectively. Older adults had significantly smaller amygdalar volumes than younger adults and there was a trend for men to have a larger left amygdala than women (see Table 3). There was no main effect of cortisol on amygdalar volume; however, cortisol output moderated the relationship of age on left and right amygdalar volume (see Table 3). The interaction of age and cortisol output in Model 3 explained an additional 5.7% and 6.9% of variance in left and right amygdalar volume, respectively, beyond that contributed by Model 1 and 2. To decompose this interaction, we obtained simple intercepts, simple slopes, and regions of significance using the online program written by Preacher, Curran, and Bauer (http://www.quantpsy.org/interact/mlr2.htm). We assessed cortisol output as the moderator at +1 SD (i.e., higher) and -1 SD (i.e., lower) from the mean. The simple slope for higher cortisol output was negative and significant for amygdalar volume in the left (simple slope = −9.26, $p < .001$) and right (simple slope = −9.14, $p < .001$) hemisphere, while the slopes for lower cortisol output were not significant (see Fig. 2). These results illustrate that older adults with higher cortisol output had smaller amygdalae than younger adults with the same cortisol output; however, at lower cortisol output, older and younger adults had similar amygdalar sizes. We determined regions of significance for simple slopes of age and found that these slopes were significant beyond 54 and 59 years of age, respectively, for the left and right amygdala (left amygdala: simple slope at region boundary = −2.52, $p = .05$ and at mean age + 1 SD = −4.79, $p = .02$; right amygdala: simple slope at region boundary = −2.83, $p = .05$ and at mean age + 1 SD = −4.55, $p = .02$). These results suggest that middle aged and older adults with higher cortisol output had smaller amygdalar volumes than same-aged adults with lower cortisol output and that amygdalar sizes in younger adults were similar regardless of cortisol output. Significant relationships were not found for interactions involving the linear or quadratic cortisol slope; neither moderated the relationship of age to amygdalar size.

In secondary analyses with the addition of depression symptoms, time interval between cortisol and MRI, and health-related covariates (i.e., BMI, cigarette smoker, hypertension, and diabetes), cortisol output remained a significant moderator of the relationship between age and left and right amygdalar size (see Table 4).

3.4. Rostral anterior cingulate cortex (ACC) thickness

Model 1 containing age and sex was not a significant predictor of left or right rostral ACC thickness. The addition of cortisol in Model 2 did not explain variance in left or right rostral ACC thickness. Neither cortisol output nor slope (linear or quadratic) moderated the relationship of age on left or right rostral ACC thickness in Model 3.

3.5. Caudal anterior cingulate cortex (ACC) thickness

Model 1 containing age and sex was not a significant predictor of left or right caudal ACC thickness; however, there was a trend for older adults to have a thicker right caudal ACC than younger adults (see Table 3). The addition of cortisol in Model 2 did not explain any additional variance in caudal ACC thickness; however, the linear cortisol slope in Model 3 did moderate the relationship of age on right caudal ACC thickness (see Table 3). The interaction of age and linear cortisol slope explained 9.9% of the variance in right caudal ACC thickness. To decompose the Age X Linear Cortisol Slope interaction, we obtained simple slopes, simple intercepts, and regions of significance. We measured linear cortisol slope as the moderator at -1 and +1 SD from the linear cortisol slope mean, reflecting respectively a more negative cortisol slope (i.e., healthier) and a less negative (i.e., flatter and less healthy) cortisol slope. The simple slope for the healthier cortisol slope

Table 2
Correlation coefficients describing relations between age, sex, cortisol, and limbic system structure[a].

	1	2	3	4	5	6	7	8	9	10	11	12
1. Age	–											
2. Sex (0 = female)	.07	–										
3. Cortisol output	.20	.13	–									
4. Linear cortisol slope	−.05	−.01	−.59***	–								
5. Quadratic cortisol slope	−.15	−.20	−.40**	–	–							
6. L hippocampal volume	−.63**	.13	.02	−.006	.14	–						
7. R hippocampal volume	−.66**	.14	−.08	.02	.21	.89**	–					
8. L amygdalar volume	−.48***	.20	−.22	.15	.23	.63**	.71**	–				
9. R amygdalar volume	−.47**	.14	−.18	.002	.10	.67**	.74**	.77**	–			
10. L rostral ACC thickness	−.08	−.07	.13	.06	−.07	.07	.05	.09	−.05	–		
11. R rostral ACC thickness	−.01	.02	.25	−.16	−.04	.15	.17	.27	.10	.50**	–	
12. L caudal ACC thickness	.05	−.11	.08	.05	−.15	−.03	−.11	−.14	−.27	.44**	.33*	–
13. R caudal ACC thickness	.23	−.16	.09	−.09	−.06	−.16	−.21	−.30*	−.25	.10	.30*	.39*

Note. ACC: Anterior cingulate cortex; L: Left; R: Right.
[a] Correlations with linear and quadratic slope are partial correlations. The quadratic slope is controlled in correlations with the linear slope and the linear slope is controlled in correlations with the quadratic slope.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Table 3
Results of hierarchical regression analyses where amygdalar volume and caudal anterior cingulate cortex (ACC) thickness are dependent variables.

	Left amygdalar volume				Right amygdalar volume					Left caudal ACC thickness				Right caudal ACC thickness			
	B	β[a]	*p*	*r*[b]	*B*	β[a]	*p*	*r*[b]		*B*	β[a]	*p*	*r*[b]	*B*	β[a]	*p*	*r*[b]
Model 1									**Model 1**								
Age	−6.78	−.50	< .001	−.51	−6.34	−.48	< .001	−.48	Age	.001	.06	.67	.06	.003	.24	.09	.25
Sex[c]	115.19	.47	.06	.27	83.96	.35	.16	.20	Sex[c]	−.05	−.23	.42	−.12	−.07	−.34	.22	−.18
Model 2									**Model 2**								
Age	−6.36	−.47	< .001	−.48	−6.04	−.46	.001	−.46	Age	.000	.03	.83	.03	.003	.24	.10	.24
Sex[c]	124.19	.51	.04	.29	90.28	.38	.14	.22	Sex[c]	−.08	−.34	.25	−.17	−.07	−.35	.23	−.18
Cort output	−1.55	−.16	.20	−.19	−1.09	−.12	.37	−.13	Quadratic slope	−1.45	−.22	.22	−.18	−.45	−.07	.68	−.06
									Linear slope	.05	.05	.76	.05	−.08	−.09	.58	−.08
Model 3									**Model 3**								
Age	−5.86	−.43	.001	−.46	−5.51	−.42	.002	−.44	Age	.000	.02	.89	.02	.003	.22	.11	.24
Sex[c]	124.25	.51	.04	.30	90.34	.38	.12	.23	Sex[c]	−.08	−.36	.21	−.19	−.08	−.39	.16	−.21
Cort output	−2.40	−.25	.06	−.28	−1.99	−.21	.11	−.23	Quadratic cort slope	−1.19	−.19	.32	−.15	−.09	−.02	.93	−.01
Age X Output	−.13	−.26	.047	−.29	−.14	−.28	.04	−.31	Linear cort slope	.03	.03	.84	.03	−.10	−.12	.46	−.11
									Age X Linear slope	−.01	−.22	.14	−.22	−.01	−.33	.02	−.33
Model 1									**Model 1**								
R^2	.29				.25				R^2	.02				.08			
$F(248)$	9.69				7.95				$F(248)$	.40				2.19			
p	< .001				.001				*p*	.67				.12			
Model 2									**Model 2**								
ΔR^2	.03				.01				ΔR^2	.06				.006			
$F_{(change)}(147)$	1.68				.82				$F_{(change)}(246)$	1.45				.16			
p	.20				.37				*p*	.25				.85			
Model 3									**Model 3**								
ΔR^2	.06				.07				ΔR^2	.04				.10			
$F_{(change)}(146)$	4.17				4.73				$F_{(change)}(145)$	2.23				5.51			
p	.047				.04				*p*	.14				.02			

Note. cort = cortisol.
[a] β equals standardized regression coefficient.
[b] *r* equals partial correlation coefficient.
[c] Sex is coded as: female = 0 and male = 1.

was positive and significant for right caudal ACC thickness (simple slope = .007, $p = .004$), while the simple slope for the less healthy cortisol slope was not significant (see Fig. 3). Thus, older adults with a healthier cortisol slope had a thicker right caudal ACC than younger adults with a similarly shaped slope. Right caudal ACC thickness was similar in older and younger adults who both had a flatter cortisol slope. We determined regions of significance for simple slopes of age and found that these slopes were significant beyond 73.5 years of age (simple slope at region boundary = −0.39, $p = .05$ and at + 1.5 SD age = -0.48, $p = .04$). These results suggest that older-old adults with healthier cortisol slopes had thicker right caudal ACC than same-aged adults with less negative or flatter cortisol slopes and that for adults younger than older-old adults, right caudal ACC thickness was similar regardless of the shape of the cortisol slope. Significant relationships were not found for interactions involving cortisol output or quadratic cortisol slope; neither moderated the relationship between age and caudal ACC thickness.

In secondary analyses with the addition of depression symptoms, time interval between cortisol and MRI, and health-related covariates (i.e., BMI, cigarette smoker, hypertension, and diabetes), the linear cortisol slope remained a significant moderator of the relationship between age and right caudal ACC thickness (see Table 4).

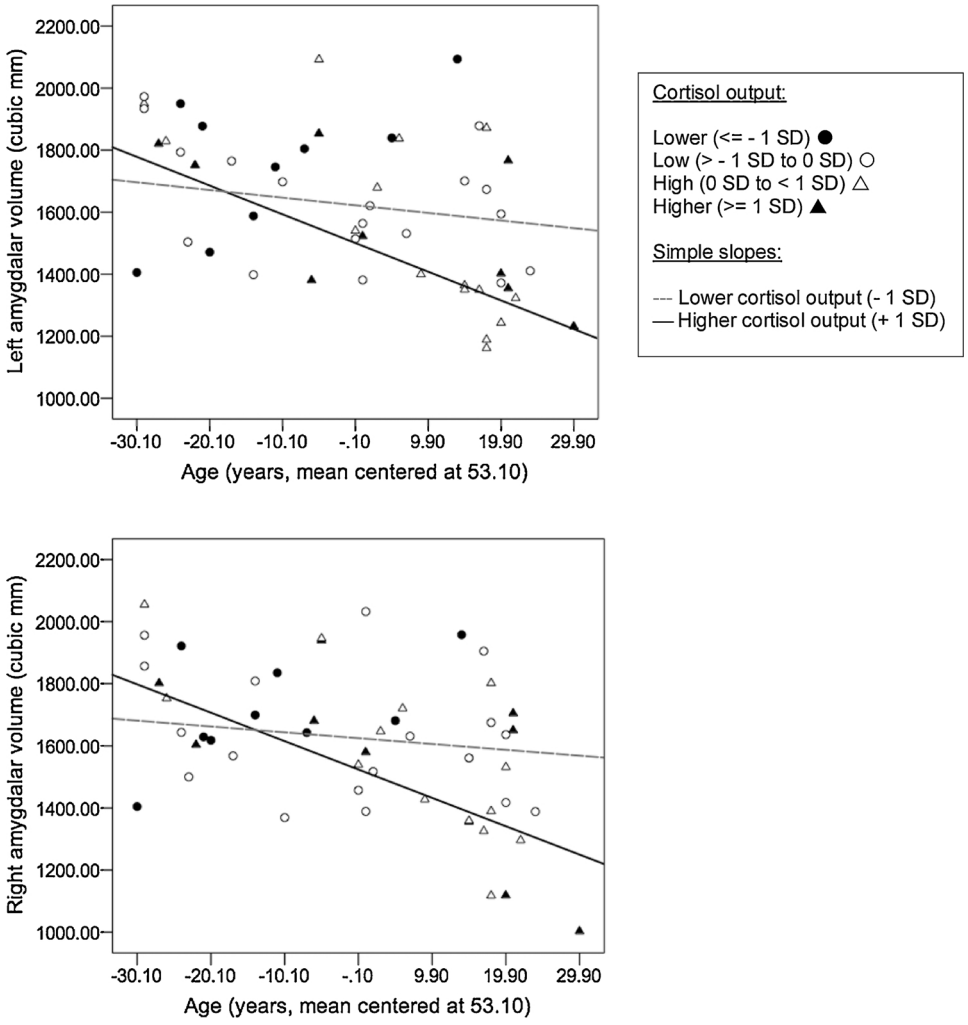

Fig. 2. Scatterplot of the relationship between age (23–83 years, mean centered at 53.10 years) and amygdalar volume with simple slopes for higher and lower levels of cortisol output.

3.6. Control: pallidum volume

Model 1 containing age and sex accounted for 32.5% and 34.3% of the variance in left and right pallidum volume, respectively. Older adults had significantly smaller pallidum than younger adults (left: $B = -10.40$, $\beta = -0.58$, $p = < .001$, $r = -0.58$; right: $B = -6.31$, $\beta = -0.60$, $p = < .001$, $r = -0.60$) The addition of cortisol in Model 2 did not explain any additional variance in pallidum volume. Neither

Table 4
Results of multiple linear regression analyses controlling for depression symptoms, health-related covariates, and time between cortisol collection and MRI.

Dependent variable →	Left amygdalar volume				Right amygdalar volume					Right caudal ACC thickness			
	B	β[a]	*p*	*r*[b]	*B*	β[a]	*p*	*r*[b]		*B*	β[a]	*p*	*r*[b]
Age	−6.14	−.45	.002	−.47	−6.12	−.46	.003	−.45	Age	.003	.24	.14	.23
Sex[c]	143.11	.58	.03	.34	84.92	.36	.20	.20	Sex[c]	−.06	−.27	.40	−.14
Depression symptoms	−3.16	−.11	.43	−.13	−3.37	−.12	.43	−.13	Depression symptoms	.001	.04	.82	.04
Diabetes[d]	−166.92	−.68	.11	−.25	2.76	.01	.98	.004	Diabetes[d]	−.08	−.40	.45	−.12
Cigarette smoker[d]	108.12	.44	.27	.17	79.60	.33	.44	.12	Cigarette smoker[d]	.01	.05	.92	.02
Body mass index	−1.52	−.03	.81	−.04	−.27	−.006	.97	−.01	Body mass index	−.001	−.04	.83	−.04
Hypertension[d]	47.99	.20	.52	.10	37.22	.16	.64	.08	Hypertension[d]	−.02	−.09	.82	−.04
Time between cortisol and MRI	6.83	.22	.13	.24	5.15	.17	.27	.17	Time between cortisol and MRI	.005	.20	.23	.19
Cortisol output	−1.74	−.18	.20	−.20	−1.14	−.12	.43	−.13	Quadratic cortisol slope	−.28	−.05	.80	−.04
Age X Output	−.15	−.29	.03	−.34	−.16	−.32	.03	−.33	Linear cortisol slope	−.12	−.14	.45	−.12
									Age X Linear slope	−.01	−.33	.045	−.32

Note. MRI: magnetic resonance imaging; ACC: anterior cingulate cortex.
[a] β equals standardized regression coefficient.
[b] *r* equals partial correlation coefficient.
[c] Sex is coded as: female = 0 and male = 1.
[d] Diabetes, cigarette smoker, and hypertension are coded as: no = 0 and yes = 1.

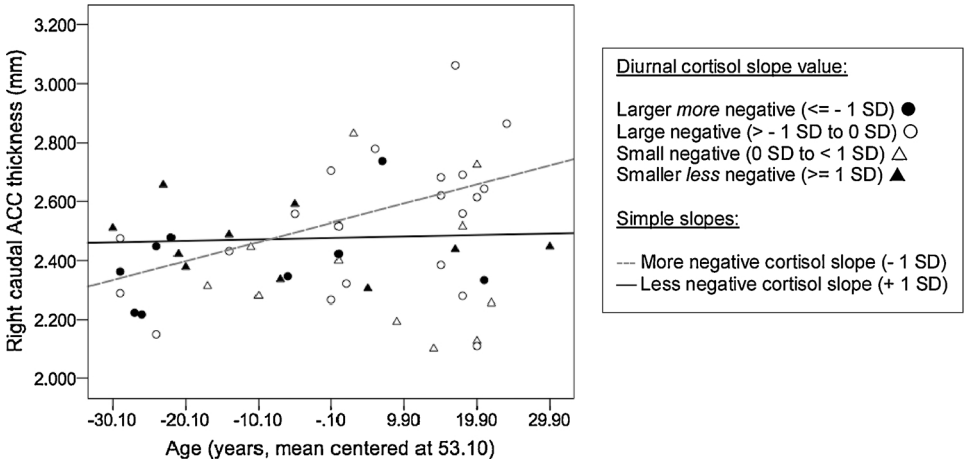

Fig. 3. Scatterplot of the relationship between age (23–83 years, mean centered at 53.10 years) and right caudal anterior cingulate cortex (ACC) thickness with simple slopes for more negative and less negative linear cortisol slope.

cortisol output nor slope (linear or quadratic) moderated the relationship of age on left or right pallidum volume in Model 3.

4. Discussion

In the present study, we replicated well-established associations between age and selected structures within the limbic system and investigated whether individual differences in cortisol output and slope may moderate these associations. The negative relationship between age and amygdalar volume was moderated by cortisol output such that middle-aged and older adults (estimated at > 54 years for left amygdala and > 59 years for right amygdala) with higher cortisol output had smaller amygdalae than same-aged adults with lower cortisol output. Further, amygdalar size was similar in older and younger adults with lower cortisol output.

Long-term exposure to increased cortisol may be one factor accounting for individual differences in age-related amygdalar volume loss. Although a longitudinal study would be needed to better test this hypothesis, results from a cross-sectional study examining the relationship between long-term corticosteroid therapy and amygdalar volume found that adults who received corticosteroids longer had smaller amygdalae (Brown et al., 2008).

Cortisol elevation due to early-life stress may not influence limbic system volumetrics until later in life. Gerritsen et al. (2015) found that self-reported high stress levels, occurring within the first 18 years of life, were associated with smaller amygdalae in older age. Although cortisol was not measured in that study, childhood disadvantage has been linked to greater cortisol output in adulthood (Franz et al., 2013); thus, elevated cortisol could be a possible mediator between early-life stress and smaller amygdalar volume in older adults. Stress response systems that become dysregulated due to early-life stress are postulated to result in allostatic load and overload, with subsequent decreases in neural resilience and increases in vulnerability to neural damage in older adulthood (McEwen et al., 2015).

However, late-life stress may have more immediate and contrasting influences on limbic system structure than early-life stress. Gerritsen et al. (2015) also found that self-report of late-life stress, occurring at age 65 or older, was related to *larger* amygdalar volumes in older adults. The apparent discrepancy between early- and late-life stress on the amygdala in humans could be accounted for by observations in animals of complex relationships between GCs/stress and amygdalar structure. Animal studies demonstrate that elevated GCs /stress increases the size of the basolateral amygdala (Vyas et al., 2002), but reduces the size of the medial amygdala (Bennur et al., 2007; Marcuzzo et al., 2007) and has no effect on the central amygdala (Vyas et al., 2003), suggesting different cortisol effects on different amygdalar nuclei. MRI at a higher spatial resolution (e.g., 7 T) than was used in the current study (i.e., 3 T) could help to resolve this issue in human populations.

The amygdala plays an important role in mediating emotional memory. It contributes to the consolidation of emotionally salient events, a process considered to be modulated by the action of GCs on the basolateral amygdala (McGaugh and Roozendaal, 2002). Although acute increases in GCs may improve emotional memory (Buchanan and Lovallo, 2001), it is not clear how prolonged elevations in GCs may influence amygdalar function and memory in older adulthood. This is an important question considering that older adults do not always demonstrate the same memory advantage for emotional material as do young adults (Charles et al., 2003) and have shown reduced amygdalar activation to negatively valenced pictures compared to younger adults (Mather et al., 2004). Whether cortisol-associated declines in amygdalae volume in older adulthood are related to emotional memory impairment deserves further investigation.

Because depression has been associated with smaller amygdalar volume (Hastings et al., 2004), we conducted secondary analyses controlling for depression symptoms as well as several health-related covariates. In these analyses, the Age × Cortisol output interaction remained a significant predictor of right and left amygdalar volume. Thus, depressive symptoms seem unlikely to account for the associations of cortisol and regional brain volume observed in the current study.

Consistent with previous studies, age was inversely related to hippocampal volume (Raz et al., 2010); however, neither cortisol output nor slope moderated the relationship of age on left or right hippocampal volume. Kremen et al. (2010) found that cortisol output, measured as the mean of five salivary cortisol samples across the day and daytime AUC, was not related to hippocampal volume in 388 middle-aged adults. Similarly, plasma cortisol levels measured in the morning and early afternoon in 95 and 88 older adults, respectively for each time period, were not related to hippocampal volume (MacLullich et al., 2005). Presumably, these studies, because of their large sample size, had adequate power to detect a significant relationship between cortisol output during the daytime and hippocampal volume. Significant relationships between cortisol and hippocampal volume have been detected in studies with much smaller sample sizes where cortisol was measured over a 24-hour period. Elevated 24-hour urinary cortisol was related to smaller hippocampal size in a combined sample of nine younger and 11 older adults (Wolf et al., 2002), and rising 24-hour plasma cortisol over time and currently-high 24-hour plasma cortisol were both related to hippocampal atrophy in 11 older adults (Lupien et al., 1998). Although further work is needed to test this assumption, 24-hour cortisol output may be essential for isolating an association between cortisol and hippocampal structure.

Age was not significantly related to the thickness of the ACC. These findings are consistent with cross-sectional studies which have reported preservation of ACC thickness (Fjell et al., 2009) and even ACC thickening (Salat et al., 2004) with increasing age. Longitudinal studies, however, have reported cingulate gyrus thinning in samples representative of the adult lifespan (Rast et al., 2017; Storsve et al., 2014). Our study suggests that cortisol regulation may be one factor related to preservation of ACC thickness in older adulthood. Older adults with a more negative linear cortisol slope (or a steeper linear decline in cortisol, indicating healthy cortisol regulation) had a thicker right caudal ACC than younger adults with a similarly shaped slope. Our moderated regression model estimated that older adults > 73.5 years of age that had a more healthy cortisol slope had a thicker right caudal ACC than same-aged adults with less healthy cortisol slopes.

That we only found a relationship with the right and not left caudal ACC suggests that the association of cortisol regulation to ACC thickness may be lateralized. Although we are not aware of studies specifically showing lateralized effects of corticosteroids on ACC anatomy, gonadal steroid exposure has been linked to cerebral asymmetry. For example, the right-greater-than-left asymmetry that is present in adult rats is eliminated by neonatal gonadectomy of male rodents (Diamond, 1991; Stewart and Kolb, 1988). Further, there appears to be functional asymmetry of the rat medial prefrontal cortex (mPFC) (Sullivan and Gratton, 1999), which is thought to be homologous with the primate ACC (Seamans et al., 2008). Lesions of the right but not the left mPFC resulted in reductions in stress-related corticosterone levels, suggesting that the right mPFC plays a greater role in stress-related glucocorticoid output.

A steeper linear decline in cortisol reflects a healthy diurnal cortisol rhythm and is associated with better psychosocial health (Adam et al., 2006). Flatter cortisol slopes have been related to disinhibition of the HPA axis as manifested in lack of suppression of cortisol following administration of the GC dexamethasone (Jarcho et al., 2013). Somewhat congruent with our findings, MacLullich and colleagues (2005) found that lack of suppression of cortisol following administration of low dose (250 μg) dexamethasone was related to smaller left ACC volume in healthy older adult men (65–70 years). This finding in conjunction with ours suggests that cortisol regulation may be associated with ACC structure in older adulthood; however, a voxel/vertex-wise analysis across the ACC would be necessary to identify which sub-regional structure is related to cortisol regulation. Additionally, whether cortisol regulation is lateralized in the human ACC deserves more investigation.

The Age x Cortisol slope interaction remained a significant predictor of right caudal ACC thickness when controlling for symptoms of depression and health-related variables. Neither depression symptoms nor any of the health variables were significant predictors of right caudal ACC thickness; thus, factors, such as diabetes and hypertension, which are more often found in older than younger adults, did not account for our findings.

These results should be interpreted in the context of study strengths and limitations. Because of the correlational nature, we cannot rule-out the possibility that small amygdalae in older adulthood may cause increased cortisol production. The amygdala plays an important role in facilitating the HPA axis response. Psychological stressors activate the medial and basolateral amygdaloid nuclei of rodents to initiate the HPA response (Ulrich-Lai and Herman, 2009). Young adult rodents with smaller basolateral nuclei produce a stronger HPA response to stress and higher elevations of GCs than young adult rodents with larger basolateral nuclei (Yang et al., 2008). If these findings are relevant to older adults, it is possible that the greater cortisol output by the older adults in our study was due to these individuals having a small amygdala. However, it would seem that this relationship would have also been found in younger adults.

We also cannot rule-out the possibility that a thicker right caudal ACC results in a healthy diurnal cortisol rhythm in older adults. The ACC contributes to the regulation of the HPA axis. Stress-related GCs act upon the ACC to dampen HPA activity, decreasing cortisol output following stressor-induced elevations (Diorio et al., 1993). Additionally, the ACC contributes to behavioral, cognitive and emotional control (Bush et al., 2000). Perhaps older adults with a thicker right caudal ACC have greater cognitive and emotional control and as a consequence a healthier diurnal cortisol rhythm. Longitudinal studies will be necessary to disentangle the relationship between cortisol regulation and ACC thickness across adulthood.

We did not have information regarding menstrual cycle phase, which may influence stressor-induced cortisol output (Kirschbaum et al., 1999). In our sample, there was not a significant relationship between sex and cortisol (see Table 2). To interrogate if the relationship between sex and cortisol was different across age, we examined the relationship of an Age X Sex interaction to cortisol. This interaction was not a significant predictor of either cortisol output ($B = -0.21$, $p = .62$) or slope ($B = -0.002$, $p = .50$). Thus, sex in our sample was not significantly related to cortisol.

Freesurfer software was utilized to measure ACC thickness and hippocampal and amygdalar volume. Because we recognized the inherent limitations with this software, we employed operators highly trained in neuroanatomy and Freesurfer usage to inspect and correct errors in the images of each participant in a standardized manner. In our experience, such a systematic approach increases the validity of cortical thickness and volumetric measures. However, even with this careful application of Freesurfer, ACC thickness and hippocampal and amygdalar volumes should be interpreted as within-sample estimates suitable for the comparative analyses that we undertook.

Participants collected cortisol 8–38 months prior to MRI. Although cortisol was collected prior to MRI, we argue that aggregating cortisol output and cortisol slope across 10 consecutive days resulted in reliable and stable measures appropriate for predicting brain structure at a later time-point. At least 4–8 days of cortisol data are needed to assess cortisol AUC reliability (Segerstrom et al., 2014); the majority of our participants had 10 days of cortisol data and no participant had less than 6 days of data. We measured potential confounds of the relationship of cortisol to limbic system structure (e.g., BMI, diabetes, and hypertension) at the time of MRI and controlled for these in secondary analyses. None of these factors was a significant predictor of hippocampal or amygdalar volume or ACC thickness. Age at time of MRI was accounted for in all of our analyses. Importantly, time interval between cortisol collection and MRI was not a significant predictor of left or right amygdalar volume or right caudal ACC thickness. Thus, the time interval between cortisol collection and MRI was likely not a significant confound in the present study.

Finally, we interpreted statistical significance at a p value of .05 and results were not corrected for the number of tests performed. We chose this approach because few neural regions of interest were examined as outcome variables. Our findings should be treated as suggestive and replication with a larger sample size will be necessary to examine the reliability of results.

In sum, this study suggests that in older, but not younger adults, cortisol output and regulation is related to differences in limbic system structure. Longitudinal studies are needed to examine whether cortisol dysregulation mediates the relationship of stressful life experiences to aging-related changes in limbic system structure.

Contributions

Gilda E. Ennis drafted the manuscript and analyzed and interpreted study data.

Eve-Marie Quintin contributed to the study design, interpretation of MRI data, and writing the manuscript.

Ursula Saelzler interpreted MRI data and reviewed the manuscript.

Kristen M. Kennedy interpreted MRI data, provided intracranial vault volume, and reviewed the manuscript.

Christopher Hertzog contributed to the study concept and design, reviewed the manuscript, and is the PI on the grant that funded the research.

Scott D. Moffat contributed to study design and the analysis and writing of the manuscript.

Declarations of interest

None.

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