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Effects of Variations in Daily Cortisol Pattern and Long-Term Cortisol Output on Hippocampal Subfield Volumes in the Adult Human Brain

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ABSTRACT

BACKGROUND: Animal models of adult chronic stress indicate that the cornu ammonis 1–3 (CA1–3) and dentate gyrus (DG) hippocampal subfields are most susceptible to cellular changes associated with prolonged psychogenic stressors and glucocorticoid overexposure. However, no study reported to date has examined associations between long-term cortisol output, chronic stress, and hippocampal subfield volumes in healthy adults experiencing different levels of chronic stress. The main goal of the current study was to test whether higher long-term cortisol output measured by hair cortisol concentration would be associated with atrophy of CA1–3 and DG hippocampal subfields.

METHODS: We examined associations between short- and long-term cortisol output and hippocampal subfield volumes in healthy adults (N = 40). High-resolution structural magnetic resonance imaging datasets were acquired together with diurnal salivary cortisol and hair cortisol measures. Hair cortisol concentration was analyzed using the high-resolution liquid chromatography–mass spectrometry method.

RESULTS: Higher hair cortisol concentration was associated with smaller volumes of all hippocampal subfields in the anterior hippocampus and smaller DG volumes in both the anterior and posterior hippocampus. We found that a larger increase in morning cortisol level after awakening was associated with smaller DG and CA1–3 volumes, while a smaller decrease in cortisol level in the afternoon from awakening was associated with smaller CA1–3 volume in the anterior hippocampus. The observed associations between cortisol and hippocampal subfield volumes were not predicted by individual chronic stress levels or history of childhood trauma.

CONCLUSIONS: Our results suggest that both increased hair cortisol concentration and daily cortisol fluctuations can have a negative impact on the CA1–3 and DG subfields.

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Stress activates the hypothalamic-pituitary-adrenal (HPA) axis, resulting in the release of corticosteroid hormones. An optimal profile of the HPA axis stress response consists of low basal glucocorticoid levels, rapid and substantial stress-induced glucocorticoid secretion, and rapid recovery once stressors abate (1). While brief HPA axis activation maximizes survival potential in the face of acute or daily stressors, continuous or excessive HPA axis activation can be maladaptive and eventually lead to mental and physical health conditions (2).

Animal models of adult chronic stress indicate that the cornu ammonis 1–3 (CA1–3) and dentate gyrus (DG) hippocampal subfields are most susceptible to cellular changes associated with prolonged psychogenic stressors and glucocorticoid overexposure (3–5). Similar to animal studies, recent magnetic resonance imaging (MRI) studies that measured hippocampal subfield volumes in patients with major depressive disorder (MDD) reported smaller volumes of the CA1–3 (6,7) and DG (7) subfields. Although several MRI studies (8), including our own (9), reported negative associations between salivary cortisol levels and hippocampal volume in patients with MDD, the majority of MDD studies with individuals on antidepressant therapies did not find such associations (10–14), suggesting potential protective effects of antidepressants on both the hippocampus (15) and HPA axis (16). In contrast, in healthy control participants, inverse associations between cortisol level and hippocampal volumes have been confirmed in many studies (9,14,17,18).

The impact of cortisol on the brain is more likely to involve continuous patterns of secretion (8). Single measures of cortisol are inadequate indices of HPA function, whereas the cortisol awakening response (CAR) better reflects the underlying physiology with timed measures at intervals after awakening (19). An elevated CAR has been associated with both acute (20) and chronic (21) stress.

Flattened diurnal cortisol slopes (DCSs), or lower degree of change in cortisol level from morning to evening over a day, has been shown to be the best predictor of poor health outcomes by a meta-analysis (22). Therefore, it may also be a

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cortisol measure that links the negative impact of stress on HPA axis and the brain. However, the associations between DCSs and hippocampal volumes in nonclinical populations have not been investigated to date.

In addition, increased long-term cortisol secretion, which reflects the cumulative burden of frequent HPA axis activation and/or long-term changes to basal cortisol secretion, is associated with a range of maladaptive effects (23). Because cortisol is incorporated into growing hair, hair cortisol concentration (HCC) reflects integrated cortisol secretion over periods of several months (24). Previous studies have reported increased HCC in endurance athletes, shift workers, unemployed individuals, patients with pain, and students with major life events (25). Studies of patients with mood and anxiety disorders have reported inconsistent findings (25), suggesting possible effects of treatment and potentially different phases in trauma exposure and processing (26). Therefore, investigating the effect of HCC in a population without a history of mental disorders would potentially disambiguate the effects of chronic stress from those of diagnosis and treatment on the HPA axis and brain structure.

Previous MRI studies that investigated associations between HCC and hippocampal volumes were conducted in children under the age of 10 years (27,28). These studies reported negative association between HCC and CA3 and DG volumes (27), CA3-DG volume (28), as well as total hippocampal volume in children with behavioral problems (29). However, no study reported to date has examined associations between HCC and hippocampal subfield volumes in healthy adults experiencing different levels of acute and chronic psychological stressors.

Hippocampal subfield distribution varies along anteroposterior hippocampal axis: A larger CA1–3 volume is located in the anterior hippocampus (head), while a bigger part of the DG is found in the hippocampal body (30). This may result in increased vulnerability of the anterior hippocampus to the effects of glucocorticoids (9). Previous MRI studies have also suggested that the adverse effects of psychological stress and childhood adversity are more pronounced in the anterior hippocampus (31) and the CA2–3 and CA4-DG subfields (32).

Therefore, the main goal of the current study was to test whether higher long-term cortisol output measured by HCC would be associated with atrophy of specific hippocampal subfields that showed cellular changes associated with glucocorticoid overexposure in preclinical studies. We hypothesized that increased HCC level would be associated with smaller CA1–3 and DG volumes in the anterior hippocampus. The second goal was to determine whether diurnal fluctuations in morning or afternoon cortisol levels measured by CAR and DCSs would be associated with increased HCC and hippocampal atrophy. We hypothesized that larger increases in morning cortisol level from awakening measured by larger CAR and smaller decreases in cortisol in the afternoon measured by flattened DCSs would be associated with increased HCC and reduced CA1–3 and DG volumes. Lastly, we aimed to test whether higher levels of stress would be associated with hippocampal atrophy. We hypothesized that increased chronic stress levels would be associated with smaller hippocampal volumes.

METHODS AND MATERIALS

Study Participants and Clinical Assessments

A total of 40 healthy participants from the general population were recruited for this study and completed behavioral assessment, cortisol tests, and an MRI scan (14 men and 26 women, ages 19–55 years). Four participants who had hair that was either too short to collect or who had <10 mg when weighed during preparation were excluded from HCC analysis. All participants had no lifetime or current psychiatric disorders as assessed by a structured interview [Anxiety Disorders Interview Schedule-V (33)] and did not meet medical exclusion criteria (see the Supplement). Written informed consent was obtained, and the research protocol was approved by the University of Alberta Health Research Ethics Board.

Distress, anhedonia, negative affect, and somatic anxiety symptoms were self-rated on the Mini Mood & Anxiety Disorders Symptom Questionnaire (34) which estimates the tripartite dimensions of anxiety and depression: general distress, anhedonic depression, and anxious arousal (35).

Assessment of Daily Stressors, Chronic Stress, and Childhood Maltreatment

Recent daily stressors were measured on the Hassles and Uplifts Scale (36). The Hassles and Uplifts Scale is a 53-item questionnaire that asks individuals to evaluate positive and negative experiences that occur in everyday life. Chronic stressors (last 3 months) were rated on the Trier Inventory for Chronic Stress (TICS) (37), a standardized 57-item questionnaire for assessing 9 factors of chronic stress (38). Perceived stress was assessed with the Perceived Stress Scale, which measures the extent to which participants felt life situations to be stressful over the last 4 weeks (39). We measured childhood maltreatment using the Childhood Trauma Questionnaire (CTQ) (40), which assesses physical, sexual, and emotional abuse and emotional and physical neglect prior to age 18. To evaluate associations between hippocampal volumes and chronic stress in the current study, we included participants with both high and low TICS and CTQ scores.

MRI Data Acquisition and Analysis

All MRI datasets were acquired on a 3T Siemens Prisma scanner (see the Supplement). MRI datasets were collected within 1 week of behavioral assessment and collection of salivary and HCC measures.

The program ITK-SNAP was used to trace hippocampal subfields, subregions, and intracranial volumes using reliable segmentation protocols (30). The hippocampus was manually segmented along the anteroposterior axis into the CA1–3, the DG, and the subiculum (Sub) subfields (Figure 1) by a single rater (JS) who was trained by the developer of the protocol (NM). Inter/intrarater reliability intraclass correlation coefficients for long-axis subregions and hippocampal subfields were estimated on a set of 10 measures per structure (Table S1).

Raw hippocampal volumes were normalized using the formula for volume correction: Normalized hippocampal volume = raw region of interest volume (mm^3)/intracranial volume of the same participant (cm^3) $\times$ 1000 (cm^3). The measures from left

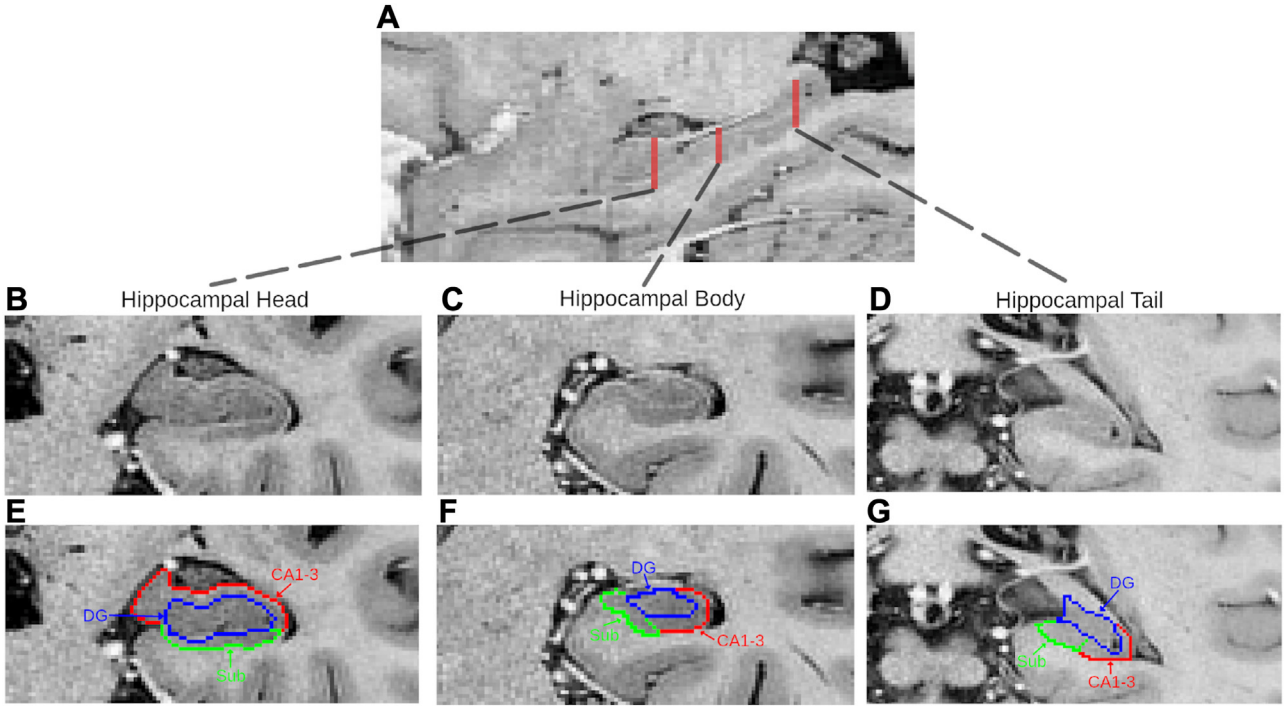

Figure 1. Segmentation of the hippocampal subfields within anteroposterior hippocampal subregions **(A–D)** is shown on T2-weighted fast spin echo images with inverted contrast: coronal views of the hippocampal head **(B)**, hippocampal body **(C)**, and hippocampal tail **(D)**. Cornu ammonis 1–3 (CA1–3) is shown in red, dentate gyrus (DG) is shown in blue, and subiculum (Sub) is shown in green.

and right hippocampal volumes were averaged for final analysis.

Salivary Cortisol Measures

During their visit, participants were given a saliva sampling kit (5 Salivettes; Sarstedt) with detailed written instructions (see the Supplement). Samples were collected over the course of 1 day at t = 0 (S1 at the time of awakening), t = 30 minutes (S2 at 30 minutes after awakening), t = 45 minutes (S3), t = 4 hours (S4), and t = 8 hours (S5). Cortisol was determined by high-sensitivity enzyme-linked immunosorbent assay (Salimetrics) with a minimum detection limit of 0.003 μg/dL. Interassay and intra-assay coefficients of variation were calculated as 5.7% (n = 5) and 5% (n = 8), respectively.

Hair Cortisol Measures

Hair samples (3-cm segments) were cut as close as possible to the scalp from a posterior vertex. A minimum of 10 mg and a maximum of 30 mg of hair was required for analysis. Based on an average hair growth rate of 1 cm/month (24), HCC in these hair segments reflects cumulative cortisol secretion over the previous 3-month period. Analysis of HCC was performed at the Mass Spectrometry Facility using our newly developed high-resolution liquid chromatography–mass spectrometry method (see the Supplement), which provides unmatched accuracy in determination of HCC measures in humans (41,42). Raw salivary cortisol and hair cortisol values were log-transformed to reduce the skewness of their distribution.

Statistical Analysis

Descriptive and inferential statistics were calculated using IBM SPSS Statistics. Awakening (S1), 30-minute (S2), and 45-minute (S3) cortisol values (Figure 2) were used to calculate the area under the curve with respect to increase (AUCi) using a formula $AUC_i = \left(\sum_{i=1}^{n-1} \frac{(m_{i+1} + m_i)}{2} \right) - (n-1)\, m_1$ described in (43). In case of negative results of the AUCi formula, showing a decrease in cortisol level (n = 22) (Figure 2), these measures were not included in the analysis of associations between AUCi and MRI measures. However, because this may result in the loss of valuable information, we conducted an exploratory statistical analysis with negative values as a cortisol index of decrease (CID) (43). We also calculated DCSs to 8 hours as the absolute change in cortisol from immediately upon waking (S1) to evening (8 hours, S5) (Figure 2). Pearson correlation coefficients (rs) were used to determine relationships between cortisol measurements, stress, clinical symptoms, and MRI measures. For correlation analysis, 2-tailed p values $< .05$ were considered significant, with Bonferroni correction used to test the associations between the cortisol for each hippocampal subfield and subregion.

To determine the power of our study sample of 36 for HCC, a conservative power estimation was performed based on previously published findings on salivary cortisol and hippocampal subfield volumes (9); to detect medium (r^2 = 0.15) to high (r^2 = 0.25) effect sizes, our study would have to have an estimated power value between 0.67 and 0.91 to detect significant associations in total CA1–3 volume.

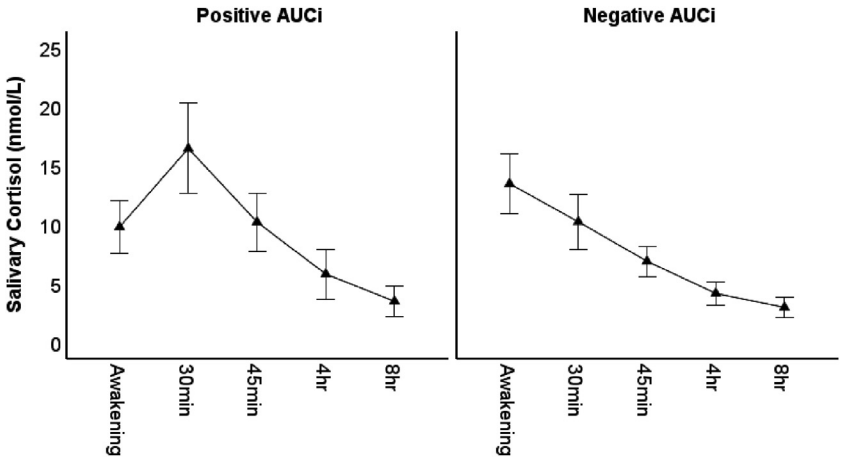

Figure 2. Mean (±2 SE) postawakening cortisol values for participants with positive and negative area under the curve with respect to increase (AUCi) values.

To account for the collinearity between some volumetric measures (see the Supplement), we also conducted a stepwise linear regression analysis. Stepwise regression for HCC level and 3 subfields/subregions (dependent variables) was used to determine associations between HCC and hippocampal volumes. Based on the significant findings from this analysis, the confirmatory stepwise regression analysis was performed to determine whether the volumetric reductions in specific subfields/subregions were present across the entire hippocampal formation, an affected specific hippocampal subregion, or a single subfield in a hippocampal subregion. A similar approach was used to test associations between DCSs to 8 hours, stress levels, and hippocampal volumes. We did not perform stepwise regression for AUCi and CID due to reduced sample size after the group was split based on positive/negative AUCi.

RESULTS

Associations Between Stress, Salivary Cortisol, and HCC

Demographics and descriptive statistics are shown in Table 1. Daily stressors (Hassles and Uplifts Scale hassles score), perceived stress level (Perceived Stress Scale score), and childhood trauma (CTQ score) were not significantly associated with salivary cortisol measures or HCC (all $ps > .14$). Total chronic stress level (TICS score) was not significantly associated with salivary cortisol measures (all $ps > .325$). We did not find significant associations between HCC and chronic stress level ($p = .68$). HCC level was not associated with any salivary cortisol measures (all $ps > .34$). Anxious arousal, anhedonic depression, and general distress level were not related to HCC (all $ps > .64$).

Relationship Between Long-Term Cortisol Output and Hippocampal Subfield Volumes

Higher HCC was associated with significantly smaller total hippocampal volume, hippocampal head volume, CA1–3, DG, and Sub volumes (Table 2 and Figure 3). After performing stepwise regression analysis to control for covariance between subfields, the average volume of CA1–3 was still found to be significantly associated with HCC in the model, while average DG and Sub volumes were no longer significantly associated with HCC (Table 3). Regression analysis also confirmed that the hippocampal head was significantly associated with HCC in the model, while average body and tail were not.

Next, we conducted confirmatory analysis of each subfield across/within 3 hippocampal subregions to determine whether these associations were present primarily in the anterior hippocampus as we previously found (9) or were present along the entire structure. When each cellular subfield was analyzed across 3 hippocampal subregions, volumes of CA1–3, DG, and Sub in the hippocampal head were found to be significantly associated with HCC in 3 separate stepwise regression models (Table 3).

When 3 different subfields in each hippocampal subregion were entered in stepwise regressions, DG volumes in the

Table 1. Participant Characteristics

	Total Sample	Men	Women	$F_{1,38}$	p
Age, Years	26.9 (8.9)	26.6 (6.5)	27.1 (10.2)	0.017	.896
Number of Participants	40	14	26	–	–
Education, Years	16.5 (1.6)	16.3 (1.9)	16.5 (1.6)	0.227	.636
Daily Hassles, HUS	42.7 (15.9)	43.4 (18.7)	42.4 (14.5)	0.033	.856
Daily Uplifts, HUS	58.2 (20.8)	60.7 (22.3)	56.7 (20.3)	0.339	.564
PSS Score	22.2 (3.2)	22.1 (3.9)	22.2 (2.7)	0.023	.881
Total CTQ Score	39.2 (13.3)	35.0 (7.8)	41.5 (15.1)	2.22	.144
Total Chronic Stress Level, TICS	22.2 (8.3)	22.6 (9.2)	21.9 (7.9)	0.06	.807
General Distress	17.6 (6.5)	17.4 (8.6)	17.7 (5.3)	0.011	.919
Anxious Arousal	13.1 (3.5)	14.6 (4.5)	12.2 (2.5)	4.51	.04*
Anhedonic Depression	22.3 (4.9)	23.6 (6.1)	21.7 (4.2)	1.39	.244
Total Mini-MASQ	52.9 (11.7)	55.6 (16.1)	51.5 (8.5)	1.076	.306

Values are presented as mean (SD) or n.

*Significant between-group differences ($p < .05$).

CTQ, Childhood Trauma Questionnaire; HUS, Hassles and Uplifts Scale; Mini-MASQ, Mini Mood & Anxiety Disorders Symptom Questionnaire; PSS, Perceived Stress Scale; TICS, Trier Inventory for Chronic Stress.

Table 2. Associations Between Diurnal Cortisol Measures, HCC, and Hippocampal Subfield Volumes

	Salivary Cortisol Level			Hair Cortisol
	AUCi	CID	DCS8h	HCC
Total Hippocampal Volume	−0.483 (p = .068)	0.496 (p = .019*)	−0.083 (p = .626)	−0.548 (p < .001[a,*])
Cornu Ammonis 1–3	−0.469 (p = .078)	0.550 (p = .008[a,*])	−0.289 (p = .083)	−0.458 (p = .006[a,*])
Dentate Gyrus	−0.548 (p = .035*)	0.317 (p = .151)	−0.094 (p = .580)	−0.439 (p = .008[a,*])
Subiculum	−0.415 (p = .140)	0.158 (p = .483)	−0.040 (p = .819)	−0.407 (p = .015[a,*])
Hippocampal Head	−0.235 (p = .4)	0.325 (p = .140)	−0.290 (p = .082)	−0.519 (p = .001[a,*])
Hippocampal Body	−0.161 (p = .55)	0.347 (p = .113)	0.169 (p = .316)	−0.314 (p = .062)
Hippocampal Tail	−0.652 (p = .008[a,*])	0.376 (p = .084)	0.124 (p = .463)	−0.186 (p = .278)

*p < .05.
AUCi, area under the curve with respect to increase; CID, cortisol index of decrease; DCS8h, diurnal cortisol slope to 8 hours; HCC, hair cortisol concentration.
[a]Significant after Bonferroni correction.

hippocampal head and body were significantly associated with HCC (Table 3).

Diurnal Cortisol and Hippocampal Subfield Volumes

We found significant negative associations between the AUCi and volumes of the DG subfield and hippocampal tail (Table 2). In contrast to negative associations between AUCi and hippocampal measures, exploratory analysis of CID showed significant positive associations with CA1–3 volume and total hippocampal volume (Table 2). Finally, DCSs to 8 hours showed only a trend toward a significant negative association with CA1–3 volume (Table 2).

Analysis of the CA1–3 and DG subfields across the hippocampal longitudinal axis revealed that AUCi also negatively correlated with CA1–3 volume in the hippocampal tail (r = −0.632, p = .015). Furthermore, CID correlated with CA1–3 volumes in the body (r = 0.436, p = .04) and tail (r = 0.472, p = .027). Analysis of CA1–3 volumes across the hippocampal subregions revealed that DCSs to 8 hours was negatively associated with CA1–3 volume in the hippocampal head (r = −0.339, p = .043).

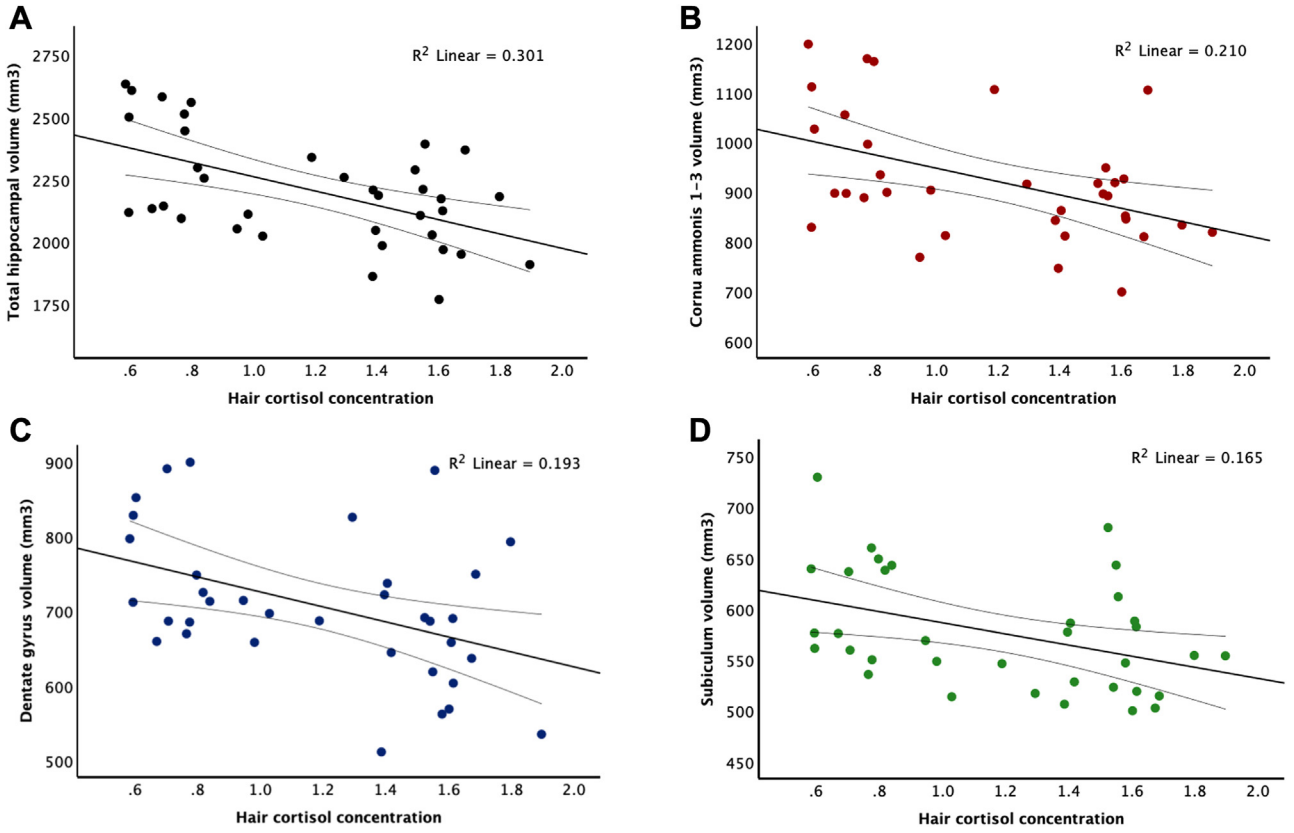

Figure 3. Associations between hair cortisol concentration and hippocampal volumes: **(A)** total hippocampal volume, **(B)** cornu ammonis 1–3, **(C)** dentate gyrus, and **(D)** subiculum.

Table 3. Stepwise Regressions on Hair Cortisol Concentration and Average HC Volumes

Variables Inputted	Selected in Model	Variable *p* Value	Excluded From Model	Variable *p* Value	Model-Adjusted R^2
HC Head HC Body HC Tail	HC head	.001	HC body HC tail	.080 .241	0.248
DG CA1–3 Sub	CA1–3	.006	DG Sub	.089 .164	0.186
Head DG Head CA1–3 Head Sub	Head DG	<.001	Head CA1–3 Head Sub	.315 .263	0.327
Body DG Body CA1–3 Body Sub	Body DG	.033	Body CA1–3 Body Sub	.799 .930	0.104
Tail DG Tail CA1–3 Tail Sub	None	–	Tail DG Tail CA1–3 Tail Sub	–	–
Head CA1–3 Body CA1–3 Tail CA1–3	Head CA1–3	.008	Body CA1–3 Tail CA1–3	.354 .562	0.168
Head DG Body DG Tail DG	Head DG	<.001	Body DG Tail DG	.326 .285	0.327
Head Sub Body Sub Tail Sub	Head Sub	.018	Body Sub Tail Sub	.407 .164	0.132

CA1–3, cornu ammonis 1–3; DG, dentate gyrus; HC, hippocampal; Sub, subiculum.

Finally, we tested whether hippocampal atrophy was directly associated with increased levels of stress. Overall, total hippocampal volume, total DG and CA1–3 volumes, or any hippocampal subregions were not significantly associated with number of daily stressors, chronic stress level, or childhood trauma (all *p*s > .28). However, smaller total Sub volume ($r = -0.347$, $p = .031$) and Sub volume in the hippocampal head ($r = -0.376$, $p = .018$) (Table 4) were associated with an increased number of daily stressors. Similarly, smaller volumes of the hippocampal body ($r = -0.335$, $p = .035$) and DG subfield with it ($r = -0.368$, $p = .021$) were associated with higher levels of perceived stress (Table 4).

Table 4. Stepwise Regressions on Average HC Volumes and Stress Measures

Dependent Variables	Independent Variables Selected in Model	Variable *p* Value	Model-Adjusted R^2
HC Volumes on Stress Measures			
Total HC volume	None	–	–
HC head	None	–	–
HC body	PSS score	.035	0.089
HC tail	None	–	–
DG	None	–	–
CA1–3	None	–	–
Sub	HUS score	.031	0.097

Variables Inputted	Selected in Model	Variable *p* Value	Excluded From Model	Variable *p* Value	Model-Adjusted R^2
HC Body Subfields on PSS Score					
Body DG Body CA1–3 Body Sub	Body DG	.021	Body CA1–3 Body Sub	.706 .617	0.112
Sub Volumes on the HUS Score					
Head Sub Body Sub Tail Sub	Head Sub	.018	Body Sub Tail Sub	.576 .234	0.118

Dependent variables include total HC volume, 3 subregions (head, body, tail), 3 subfields (CA1–3, DG, Sub). Independent variables include daily hassles score from HUS, PSS score, and Trier Inventory for Chronic Stress score. Follow-up stepwise regression on 1) HC body subfields and PSS score; 2) Sub subfield volumes and HUS scores.

CA1–3, cornu ammonis 1–3; DG, dentate gyrus; HC, hippocampal; HUS, Hassles and Uplifts Scale; PSS, Perceived Stress Scale; Sub, subiculum.

DISCUSSION

The current study demonstrated for the first time how both long-term cortisol levels and diurnal cortisol fluctuations are associated with volumetric reduction of hippocampal subfields in healthy adults. First, our results demonstrated that increased long-term HPA axis activity measured by higher HCC was associated with smaller global hippocampal volume, reduced volumes of all hippocampal subfields in the anterior hippocampus, and smaller DG volumes in both the anterior and posterior hippocampus. Second, we found that a larger increase in morning cortisol level (AUCi) in the first hour after awakening was associated with smaller DG and CA1–3 volumes. Third, the current study showed that a smaller decrease in cortisol level in the afternoon from awakening (flatter DSCs to 8 hours) was associated with smaller CA1–3 volume in the anterior hippocampus. Finally, the observed associations between diurnal cortisol and HCC and hippocampal subfield volumes were not explained by individual chronic stress levels or history of childhood trauma.

Our findings suggest that both long-term and short-term HPA axis overactivation could be associated with volumetric reduction in CA1–3 and DG subfields. The number of previous studies that investigated associations between HCC and hippocampal volumes is still small, and these studies were conducted with children. Both the amygdala and the hippocampus are regarded as targets of childhood adversity because they exhibit protracted postnatal development, a high density of glucocorticoid receptors, and postnatal neurogenesis (44,45). Chen *et al.* (29) observed a negative association between HCC and hippocampal volume in children with behavioral problems but not in typically developing children. Merz *et al.* (27) found that high child HCC was associated with smaller CA3 and DG volumes. Keresztes *et al.* (28) also found that higher HCC was related to lower CA3-DG volumes in children. In the current study, a history of childhood trauma was not related to hippocampal subfield volumes as we had previously found in patients with MDD (46). It is important to mention that the values of the CTQ scores in our healthy participants were lower than those of patients with MDD from our previous study. Our results indicate that while increased HCC was associated with volumetric reduction in total hippocampal volume and total volume of its subfields, we found fewer associations between diurnal cortisol fluctuations and hippocampal volumes, further emphasizing the vulnerability of hippocampal subfields to prolonged glucocorticoid secretion. In the current study, r^2 values for associations between HCC and total subfield volumes were in the range of 0.17 for total Sub and 0.21 for total CA1–3 volumes, indicating medium to high effect sizes. Therefore, the sample size would need to be higher to detect smaller to medium effect sizes for the subfield-cortisol associations.

The Sub is a primary mode of hippocampal interactions with the HPA axis (47,48); like the hippocampus proper, the ventral Sub expresses high levels of glucocorticoid receptors, and it is critical for stress responsiveness, whereas the dorsal component may gate information that concerns basal secretory patterns (48,49). Our results are consistent with these studies and indicate that the ventral Sub may be affected by increased long-term cortisol levels. Interestingly, in the current study, Sub volume in the anterior hippocampus was negatively associated with an increased number of daily stressors, further supporting a link between this structure and stress responsiveness.

Although HCC was not directly associated with salivary cortisol measures, our findings may provide insights into potential mechanisms of glucocorticoid neurotoxicity. In our previous MRI study (9), relative changes in salivary cortisol level from awakening to 8 hours negatively correlated with CA1–3 volume in patients with MDD, while in healthy participants, mean cortisol levels negatively correlated with CA1–3 volume. Our current findings confirmed our previous results. This study also expands on our previous findings by linking both diurnal cortisol changes and long-term cortisol output to CA1–3 and DG atrophy.

Most previous MRI studies on cortisol and hippocampal volumes were conducted in a clinical population including individuals with MDD (8,50) and posttraumatic stress disorder (51). The most consistent finding in MDD (8) was that an association between higher cortisol levels and smaller hippocampal volumes arises from repeated measures of cortisol levels over a day rather than a single measure taken at one time of the day only. Previous studies of healthy participants have found negative correlations between total hippocampal volume and 24-hour urinary free cortisol (14,18), basal corticotropin (adrenocorticotropic hormone) levels (18), plasma cortisol levels (52), and postchallenge salivary cortisol levels (17). More recent studies tested whether differences in CAR are associated with hippocampal atrophy (53–55). These studies found that although CAR was positively associated with stress (20,21,56), lower AUCi (53) and blunted CAR (54) were related to smaller hippocampal volumes in a high-risk subclinical depression group (53) and in individuals at ultra-high risk for psychosis (54), while these associations were not found in healthy individuals (53–55). In contrast to studies that used CAR, Ristanovich *et al.* (57) found that in adolescents at clinical high risk for psychosis, a greater daily stress level was associated with lower volumes of CA1, CA2/3, and CA4/DG, while higher resting cortisol was associated with lower volumes of presubiculum. Although a recent study (58) found inverse relationships between basal cortisol and hippocampal volume in healthy adolescents and in the group at clinical high risk for psychosis, life events stress was not related to hippocampal volume.

Our results indicate that the CID and DCSs to 8 hours may be 2 other diurnal cortisol measures linked to hippocampal atrophy, more specifically atrophy of the CA1–3 subfield.

Our results revealed significant positive associations between negative AUCi (CID), total hippocampal volume, and its CA1–3 subfield. This indicates that a larger decline in cortisol level from awakening in the first hour was also associated with smaller hippocampal volumes. Previous studies showed that 23% of healthy participants do not show an increase in cortisol levels at 30 minutes after awakening (59). Others have suggested that the usual CAR sampling procedure can miss the awakening rise in individuals with early morning peaks that can occur as early as 15 and 20 minutes after awakening (46,60). Therefore, CID may represent a decline from cortisol peak at awakening or from a peak that occurred at 15 or 20 minutes

after awakening. Shorter sampling times with objective methods for verification of awakening time and sampling time (61) would be required to address this study limitation directly. Because both larger increases (positive AUCi) and larger decreases (CID) in morning cortisol were associated with smaller CA1–3 volumes, they may independently represent an amplitude of cortisol rise and fall from the peak at awakening that is detectable with the current sampling methodology.

The lack of significant correlations between HCC and diurnal cortisol measures can be explained by the fact that the HCC measured in the current study represents integrated cortisol production over 3 months, while diurnal cortisol measures reflect cortisol level over the course of a single day. Consistent with the current findings, previous validation studies that measured diurnal cortisol levels over a 30-day course also did not find significant relationships between CAR (62,63), DSC (62,63), and HCC levels. Although we did not find significant associations between CID, DCSs to 8 hours, and HCC, both of these measures were linked to hippocampal subfield volumetric reduction. Consistent with these findings, a meta-analysis (64) did not find significant associations of HCC with measures of the CAR or overall postawakening cortisol secretion. They also did not observe significant associations between HCC and perceived stress (Perceived Stress Scale) or self-reported chronic stress (TICS). This is consistent with the current study. Similarly, another systematic review (25) did not find significant associations between total TICS scores and HCC. However, the TICS subscale social overload was positively associated with HCC, while other TICS subscales did not show associations with HCC (25). This may suggest that not all types of chronic stressors equally activate the HPA axis.

A recent preclinical study indicated that while glucocorticoids can diffuse from the bloodstream into hair within 3 hours, they can remain in the hair for at least 2 weeks following a massive stressor and diffuse out of the hair again (65). This may suggest a shorter time span (days/weeks) between experienced stressors and increased HCC in humans than is currently estimated for a much longer duration (1–3 months). Although our findings revealed significant negative associations between the Sub and DG subfields with daily stressors and perceived stress level, due to a relatively small sample size, we did not perform structural equation modeling to test whether the indirect effects of stress on hippocampal subfield volumes were mediated by cortisol levels.

Our findings that the CA1–3 subfield was sensitive to both increased HCC levels and diurnal cortisol fluctuations are consistent with preclinical studies that have demonstrated that the CA3 subfield was the most vulnerable to the impact of stress (66–69). Our previous study (30) demonstrated that the largest proportion of the CA1–3 subfield is in the anterior hippocampus, likely making this subregion a main target of glucocorticoid as the current study found. However, HCC also showed negative associations with the DG subfield in both the anterior and posterior hippocampus, suggesting that long-term glucocorticoid exposure may also affect neurogenesis in the DG subfield. It remains to be determined whether the reversibility of hippocampal atrophy is linked to changes in cortisol levels. In their recent longitudinal study, Valk *et al.* (70) observed increases in CA1–3 volume after 3 months of compassion-based training that correlated with decreases in total diurnal cortisol output. They also found associations between increases in left CA1–3 volume and HCC decreases. Future studies are needed to test whether this process is driven by changes in chronic ongoing stress.

Several limitations of this study should be mentioned. Our study sample was relatively small, and we did not test for sex differences. Future research on this topic should test whether these effects are stronger in women than men. Longitudinal studies are also needed to directly assess the sequence of changing cortisol levels, chronic stress, and hippocampal subfield volumes, as well as treatment effects in patients with MDD.

Conclusions

Our results suggest that both increased HCC and daily cortisol fluctuations were associated with smaller CA1–3 and DG volumes, while direct effects of chronic stress and history of childhood trauma were not significant.

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NM, JS, WP, and BR were responsible for formal analysis. NM was responsible for methodology, design, acquisition of funding, and writing the original draft of the article. NM, JS, KH, BR, WP, and RW were responsible for investigation. All authors reviewed and revised the article.

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