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Salivary Cortisol and Regional Brain Volumes Among Veterans With and Without Posttraumatic Stress Disorder

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ABSTRACT

BACKGROUND: Human studies have often found that brain regions rich in glucocorticoid receptors exhibit smaller volume in samples with past trauma and ongoing stress; however, relatively little research has addressed the hypothesis that such smaller volumes can be traced to elevated circulating glucocorticoid hormones (GCs). This issue takes on renewed interest in light of recent proposals to treat symptoms of stress disorders such as posttraumatic stress disorder (PTSD) with exogenous synthetic GCs. We sought to examine the relation of circulating GCs to brain macrostructure among veterans with and without PTSD.

METHODS: Participants ($n = 90$) included combat veterans with and without PTSD. Veterans completed self-report surveys, home-based cortisol samples, reactive cortisol samples over the course of two serial Trier Social Stress Tests, a low-dose dexamethasone suppression test, and structural magnetic resonance brain imaging over the course of 3 to 5 days.

RESULTS: No associations were observed between any salivary cortisol index and the volumes of the hippocampus or amygdala. A negative association was observed between evening basal cortisol and both FreeSurfer global volume and BrainImage supratentorial tissue volume. This effect was moderated by PTSD. Also observed was a positive association between reactive cortisol and these same brain volumes.

CONCLUSIONS: Estimates of cortical but not hippocampal or amygdala volume were moderately associated with evening basal salivary cortisol and cortisol reactivity to a social stressor. Existing models relating GC receptor density, circulating cortisol levels, and regional brain volumes received little support.

Keywords: Amygdala, Cortisol, Hippocampus, Posttraumatic stress disorder, Regional brain volumes, Veterans

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The release of glucocorticoid hormones (GCs) in response to acute stress orchestrates an array of adaptive and regulatory functions (1,2). Chronic GC elevations are associated with structural modifications in the brain, the consequences of which are still unclear. Within the hippocampus, chronic GC elevations were found to be associated with regression of CA3 pyramidal apical dendritic arbors, and even with cell death (3,4). Such results led to pioneering studies that found smaller hippocampal volume in posttraumatic stress disorder (PTSD) (5,6), findings that have been generally replicated (7–9). Studies in animals soon extended beyond the hippocampus, demonstrating that exogenous glucocorticoid administration or restraint stress resulted in similar retraction of the apical dendritic arbors in the anterior cingulate cortex (ACC) of rats (10,11). In apparent convergence, human studies have consistently found ACC cortical volume to be smaller in persons with PTSD (7,12).

While human studies have often found that brain regions rich in glucocorticoid receptors (GRs) exhibit smaller volume in samples with past trauma and ongoing stress, relatively little attention has been paid to the original hypothesis that these smaller volumes can be traced to elevated circulating GCs. Data accumulated over the past 2 decades have not strongly supported this proposition, which has taken on renewed interest in light of recent proposals to treat symptoms of stress disorders such as PTSD and major depressive disorder (MDD) with exogenous synthetic GCs (13,14). Across the psychiatric conditions in which this relationship has been assessed, including PTSD, MDD, psychosis, and chronic pain, approximately one half of published studies have found either no relationship between GC levels and regional brain volumes or positive relationships (see Supplemental Table S1 for a list of studies). In fact, two of the three studies employing PTSD samples failed to support the original hypothesis. Lindauer *et al.* (15) found that morning salivary cortisol levels were positively correlated with the volume of the right hippocampus. Neylan *et al.* (16) found no relationship between salivary cortisol and hippocampal volume, but a positive correlation between cortisol and levels of *N*-acetylaspartate, a marker of neuronal integrity, in left hippocampus.

A similar distribution of confirmatory and nonconfirmatory results characterizes studies in healthy samples and those with relevant medical conditions. In a quasi-experimental study on patients with Cushing's syndrome, Starkman *et al.* (17) found that hippocampal enlargement correlated with pharmacologically driven reductions in urinary free cortisol but not plasma cortisol. Knoops *et al.* (18) studied 575 middle-aged persons diagnosed with arterial disease and found modest negative associations between hippocampal volume and evening salivary cortisol levels, but not with an aggregate waking estimate. Kremen *et al.* (19) studied 189 monozygotic twin pairs and found that two aggregate measures of waking salivary cortisol samples (mean and area under the curve) were negatively correlated with the thickness of a number of prefrontal cortical parcels, but not with the surface areas of those same parcels, nor with the volume of the hippocampus. The latter failure could have derived from reliance on a FreeSurfer-based estimate of hippocampal volume, as these have been shown to account for only 50% of the variance of manually delineated volumes (20).

We sought to address the relation of circulating GCs to brain macrostructure in a relatively large sample of combat veterans with chronic severe PTSD and combat control subjects free (currently or lifetime) of PTSD. We have already reported on a number of associations between PTSD and regional brain volumes in this sample (12,21). Importantly, both smaller hippocampal volume and smaller ACC volume have been confirmed in the PTSD subsample compared with the trauma-exposed control subjects, though the former effect was moderated by lifetime alcohol use disorder. Using salivary cortisol, we considered basal morning and evening cortisol, cortisol suppression by dexamethasone (DEX), cortisol reactivity to a social stressor, and the habituation of cortisol reactivity to that stressor. Examination of the latter was motivated by the proposal of McEwen and Margarinos (22) that delayed habituation to repeated stressors could resolve the apparent incongruency between the depressed cortisol levels often observed in PTSD samples (23–25) and brain volumetric effects supposedly traceable to elevated GCs. We also brought to bear two sets of regional brain volume measurement, one based on manual delineation and a second based on FreeSurfer. The former offered optimal measurement of specific volumes-of-interest such as hippocampus and amygdala, while the latter offered optimal measurement of the cerebral cortex and is in wide use today. Our interest in whole-cortex coverage derived, in part, from suggestions that GRs are more widely distributed in the primate than the rodent brain (26–28). In view of the distribution of GRs to all of these regions, we hypothesized that all measures of circulating GCs—basal, reactive, nonhabituation of reactive over repeat stressors, and supersuppression by low-dose DEX—would be associated with smaller hippocampus, amygdala, ACC, and global cortical volumes.

METHODS AND MATERIALS

Participants

Participants were U.S. military veterans (female = 5.6%; M_{age} = 48.11, SD = 9.36) with (n = 45) and without (n = 45) PTSD. Individuals participated in a parent study examining regional brain volumes as a function of PTSD diagnostic status and lifetime alcohol use disorders (12). A brief overview of the methods are described below, and a more detailed overview of the methods have been described elsewhere (12). Individuals were excluded based on evidence of current or past central nervous system disease, psychosis, and alcohol or substance use disorders within the past 6 months and on known contraindications for magnetic resonance imaging. All participants reported exposure to combat-related trauma during the Gulf War (n = 33) or Vietnam War (n = 57) meeting DSM-IV defined criterion A for the diagnosis. A majority of the sample (45.6%) also experienced a criterion A trauma at age 17 years or younger. Participants with PTSD, compared with those without PTSD, had fewer years of education, were less likely to be employed, were more likely to be non-Caucasian, and were more likely to smoke. In addition, those with PTSD had higher rates of current and lifetime MDD and were more likely to be taking psychoactive medications, principally selective serotonin reuptake inhibitors, serotonin and norepinephrine reuptake inhibitors, and gamma-aminobutyric acid agonists (see Table 1).

Psychodiagnostics

Diagnoses were obtained using the Clinician-Administered PTSD Scale (29) and the Structured Clinical Interview for DSM-IV (30). Additional measures of declarative memory were obtained that are not germane to this report (31).

Home-Based Salivary Cortisol Collection and Quantification

Participants provided home-based saliva samples in the morning (8 AM) and evening (7 PM) on two consecutive days (basal cortisol). All saliva samples were obtained using prelabeled Salivettes (Sarstedt, Nümbrecht, Germany). Participants were instructed not to eat or drink (except water) for 90 minutes before sample collection and not to smoke for 60 minutes before sample collection. Participants also self-administered a low-dose DEX test over a separate 2-day period. This test consisted of an 8 AM saliva sample, ingestion of a 0.5-mg tablet of DEX at 10 PM, followed by a saliva sample, and an 8 AM sample the following morning. Salivettes were brought or express-mailed to the laboratory. Salivettes were spun at 3000 revolutions per minute to remove saliva from the swabs. Saliva samples were then pipetted into 4-mL Wheaton vials (Millville, NJ) and stored at −70°C. Samples were batch shipped over dry ice to PsychoNeuroEndocrine Research Laboratory in Louisville, KY, for analysis by radioimmunoassay. This assay used a luminescence-based variation of the standard enzyme-linked immunosorbent assay. An enzyme was used to convert a substrate, linked to cortisol within the sample, into a reaction product that emitted photons of light instead of developing a visible color (as in enzyme-linked immunosorbent assay). We used a luminescence plate reader rather than a colorimetric one. Similar to enzyme-linked immunosorbent assay, we used standards and both high and low control samples to create a standard curve and estimate the reliability of our assay. Standards are samples with known concentrations of cortisol. Control samples are large volume samples that are assayed each time we ran an assay, and the results are used for reliability estimates across multiple assays. Assay sensitivity was 0.007 μg/dL.