EARLY ADVERSE EVENTS, HPA ACTIVITY AND ANTERIOR CINGULATE VOLUME IN MAJOR DEPRESSIVE DISORDER

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CHAPTER I

INTRODUCTION

Stress, glucocorticoids and grey matter volume

Early adverse events have been shown to be a significant risk factor for the subsequent development of major depressive disorder (MDD) (Agid, Shapira, Zislin, Ritsner, Hanin, Murad et al., 1999). One theoretical pathway by which early adverse events may increase the risk of developing depression is by increased activation of stress hormones. This hypothesis is derived from two areas of research. First, both animal and clinical models suggest that depression is associated with poor regulation of HPA axis activity, as indicated by elevated cortisol (Thase, Jindal & Howland, 2002), disruption of circadian HPA rhythms (Thase, et al., 2002; Aborelius, Owens, Plotsky & Nemeroff, 1999), and failure to suppress cortisol levels following administration of the synthetic steroid dexamethasone (Aborelius et al., 1999). Secondly, preclinical studies have revealed that prolonged exposure to glucocorticoids is associated with atrophy of brain regions involved in the regulation of HPA activity, including the hippocampus (Sapolsky, 2000) and the medial prefrontal cortex (mPFC) (Diorio, Viau & Meaney, 1993). Stress-related damage to these regulatory regions may precipitate a cycle of diminished resiliency, in which the system is less able to regulate HPA activity in response to future stress, resulting in greater exposure to glucocorticoids.

Prior research has highlighted the role of the hippocampus, given its well-established role in regulating HPA activity, and high density of glucocorticoid receptors

(Sapolsky, 2000). However, more recent work has also emphasized a prominent role of the mPFC, including the anterior cingulate cortex (ACC). Findings from preclinical studies suggest that the ACC contains high concentrations of glucocorticoid receptors (GR) in laminas II, III, and V (Ahima & Harlan, 1990), is vulnerable to the noxious effects of glucocorticoids (Radley, Sisti Jao, Rocher, McCall, Hot, et al., 2004; Cerqueira, Cantania, Sotiropoulos, Schubert, Kalisch, Almeida et al., 2005), and may exert inhibitory control over the HPA axis via GR-mediated negative-feedback (Ahima & Harlan, 1990; Akana, Chu, Soriano & Dallman, 2001). In addition, corticosterone implants in rat mPFC, but not in the amygdala, lead to reduced ACTH secretion in response to acute restraint stress (Akana et al., 2001). Finally, exposure to chronic stress and elevated corticosteroid levels have been shown to precipitate dendritic pruning and volumetric decreases in the rat ACC (Radley et al., 2004; Cerqueira et al., 2005; Akana et al., 2001).

Consistent with these preclinical findings, human neuroimaging studies of individuals with MDD have reported volumetric reductions in the hippocampus (Sheline, Sanghavi, Mintun & Gado, 1999; Sheline, Gado & Kraemer, 2003; Vythilingam, Heim, Newport, Miller, Vermetten, Anderson et al., 2002; Hastings, Parsey, Oquendo, Arango & Mann, 2004; Campbell & MacQueen, 2006) and the ACC (Hastings et al., 2004; Caetano, Kaur, Brambilla, Nicoletti, Hatch, Sassi et al., 2006; Botteron, Raichle, Drevets, Heath & Todd, 2002). Post-mortem analyses have also found that the ACC in individuals with MDD exhibits diminished neuronal size and reduced glial cell density (Cotter, Mackay, Landau, Kerwin & Everall, 2001). Decreases in whole-cingulate volume have also been

associated with clinically reported early life stress (Cohen, Grieve, Hoth, Paul, Sweet, Tate et al., 2006).

Evidence for stress-related structural damage to the anterior cingulate is particularly relevant for understanding the relationship between stress and depression, as significant research suggests that cortico-limbic pathways involving the ACC may be responsible for impairments of cognition, emotion and motivation in MDD (Mayberg, 2003). Hypoactivity in the dorsal subdivision (dACC) (Ebert & Ebmeir, 1996), and elevated rostral cingulate activity (rACC) (Mayberg, 1997), have been demonstrated in MDD, along with impaired ACC-amygdala connectivity (Anand, Yu, Wang, Wu, Gao, Bukhari et al., 2005).

In the present study, we sought to examine the relationship between early adverse events, HPA activity and grey matter volume among individuals with unipolar depression and healthy controls, with a particular emphasis on grey matter volume of the hippocampus and rostral anterior cingulate. While previous studies have identified the volumetric decreases in the ACC, (both whole-volume (Caetano et al., 2006) as well as left subgenual cingulate (Hastings et al., 2004; Botteron et al., 2002), these decreases have not been linked to both early adverse events and circulating cortisol levels. By assessing all three variables within a single sample, the present study is able to provide a more direct evaluation of the putative neurobiological mechanisms that are believed to volumetric decreases of regions involved in HPA regulation in MDD.

We used voxel-based morphometry (VBM) to evaluate regional differences in grey-matter volume in a sample of individuals with MDD and a group of never-depressed matched healthy controls. Volumetric differences were evaluated using both a whole-

brain, and region of interest (ROI) approach. *A priori* ROI included the anterior cingulate (dorsal and rostral ACC [BA 24, 32 and 25]) and the hippocampus. These regions were selected because both have shown evidence of atrophy in MDD (Sheline, 1999; Sheline, 2003; Vythilingam et al., 2002; Hastings et al., 2004), and preclinical findings have suggested that both regions are involved in regulating HPA activity (Sapolsky, 2000; Diorio et al., 1993), and are damaged by elevated exposure from glucocorticoids resulting from chronic stress (Sapolsky, 2000; Radley et al., 2004; Cerqueira et al., 2005; Akana et al., 2001). In addition, we collected self-report data on history of childhood trauma, and acquired samples of salivary cortisol. We hypothesized that depressed individuals would show decreased volume in the ACC and hippocampus when compared to controls, and that these volumetric decreases would correlate with a history of reported early adverse events as well as cortisol levels.

CHAPTER II

METHODS

Participants

The experimental protocol was approved by the Vanderbilt University Institutional Review Board. A complete description of the study was provided to all participants, and all subjects provided written informed consent. Subjects were recruited through the Vanderbilt University Medical Center Outpatient Psychiatry Clinic or through television advertisements. Participants were between 18 and 55 years of age with no significant history of neurological disease or lifetime history of brain injury, psychosis, mania, substance dependence or substance abuse in the past six months. All patients were diagnosed with unipolar depression and met full criteria for one or more episodes of major depressive disorder as determined by a Structured Clinical Interview (SCID) for DSM-IV. Patients were excluded if they met criteria for specific comorbid Axis I disorders that included alcohol dependence, obsessive-compulsive disorder, schizophrenia and other psychotic disorders or bipolar disorder. In addition, a score of 16 or higher on the Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960) was required. Patients were antidepressant-free at the time of scanning. Never depressed control subjects did not meet criteria for any Axis I mood disorders except for one subject who was diagnosed with mild agoraphobia without panic disorder as determined by the SCID. All never-depressed control subjects had a score of six or less on the

HDRS. Subjects who met criteria were then scheduled for a scan session within one week of admission to the study.

Table 1. Means, standard deviations and group comparisons of demographic data and HDRS scores.

Variable	MDD			Healthy Controls		
	N	*Mean*	*SD*	*n*	*Mean*	*SD*
Age	18	35.6	10.7	15	28.9	8.5
% Female	18	50%		15	60%	
Estimated IQ (Shipley)	18	105.4	8.0	15	108.1	10.9
HRSD**	18	21.7	4.1	15	0.93	1.4

*** p < .001*

Behavioral Measures

Child trauma questionnaire

To assess a history of early adverse events, participants completed the Childhood Trauma Questionnaire – Short Form (CTQ-SF) (Bernstein, Stein, Newcomb, Walker, Pogge, Ahluvialia et al., 2003). The CTQ Short Form was developed as a 28-item questionnaire derived from the original 70-item Childhood Trauma Questionnaire. The CTQ-SF has 25 clinical questions and three validity items. The measure has five sub-scales comprised of five questions each that assess childhood maltreatment in the areas of emotional, physical, or sexual abuse, emotional neglect and physical neglect. Subjects rate statements about childhood lifetime experiences on a five-point scale ("never true" to "very often true"). Items are generally stated in objective terms, (e.g., "When I was growing up, someone touched me in a sexual way or made me touch them"), whereas some items require subjective evaluation (e.g., "When I was growing

up, I believe I was sexually abused") and usually do not specify the perpetrators relationship to the subject. Emotional abuse items are general (e.g., "People in my family said hurtful or insulting things to me") but do not investigate the specific verbal content of the abuse. Reliability and validity of the CTQ, including its stability over time, convergent and discriminant validity with structured trauma interviews, and corroboration using independent data have been determined. The CTQ-SF has demonstrated high internal reliability, with (Cronbach's alpha from .74 to .90) and good test-retest reliability at three months ($r = .80$). Scores on each subscale were calculated by taking the mean value of the five individual items for each subscale. Scores of patients and controls subscales were compared using an independent samples t-test, assuming unequal variance. A Bonferroni correction was applied to control for multiple comparisons (corrected $\alpha = .01$).

Salivary cortisol

Samples of saliva were collected using the Salivette saliva collection device (Sarstedt, Newton NC). Participants collected three saliva samples per day for two consecutive days and a sample immediately prior to their MRI scan session on the third day. The first sample was recorded within 0.5 h after awakening. Two additional samples were collected at 3:00 PM and 9:00 PM. Using this method we ensured that we could adequately account for diurnal variation. Cortisol levels were determined using an enzyme immunoassay (ALPCO Diagnostics, Salem, NH). For all subsequent analyses, the average of all seven cortisol samples was used unless otherwise specified.

Neuroimaging Measures

Image acquisition

Resting-state MRI scans were acquired on a 3T Philips Intera Achieva scanner (The Netherlands) at the Vanderbilt University Institute of Imaging Sciences (VUIIS). High-resolution structural images were acquired in the axial plane to facilitate spatial normalization using a 3D IR Prepped 3DFFE sequence (TR=10.1ms, TE=4.2ms, FOV=24x24cm2, matrix size=256x256, slice thickness=l.2mm, no gap). Due to scanner error, 3D data were not available for one subject. For this subject we used a 2D imaging sequence (TR=450ms, TE=17ms, FOV=24x24cm2, matrix size=256x256, slice thickness=4mm, no gap). Inclusion of this subject did not significantly alter the results.

Voxel-based morphometry

Data were analyzed on a Dell Vostro 200 (Dell Inc, Round Rock, TX) running a Linux-based operating system (Ubuntu 7.1). Voxel-based morphometry (VBM) was performed using MATLAB7.4.0 (Mathworks, Natick, MA) and SPM2 (Wellcome Department of Imaging Neuroscience, London, UK). All VBM analyses strictly adhered to the optimized VBM protocol as described by Good et al (2001) (Good, Johnsrude, Ashburner, Henson, Friston & Frackowiak, 2001). All structural images were examined for artifacts and then reoriented to a center point located on the anterior commissure. Using the control subjects only, a customized anatomical template was created from the reoriented structural MRI images. Template creation included spatial normalization of all

the images to the same stereotactic space. The customized template was then used as the basis for spatial normalization for all subjects. Spatially normalized images were then re-sliced with a final voxel size of approximately 1.5 x 1.5 x 1.5 mm^3, which were subsequently segmented into grey matter, white matter, and cerebrospinal fluid (CSF). After segmentation, the segmented grey-matter images were modulated by multiplication of the Jacobian determinant of the spatial normalization function, so as to allow for the estimation of volumetric differences between groups (Good et al., 2001). Images were then smoothed using a 12-mm FWHM isotropic Gaussian kernel. All subsequent statistical analyses were performed on the normalized, segmented, modulated and smoothed grey matter images. Group differences between patients and controls were assessed using an ANCOVA model as implemented in SPM2, with total intracranial volume used as a covariate to control for individual differences in total volume. Total intracranial volume was calculated as the sum of segmented gray, white and CSF images for each subject. Whole-brain analyses were conducted with a voxel-wise correction for multiple comparisons using a family-wise error correction of $p_{FWE} < .05$

ROI analysis

Regions of interest were drawn for the anterior cingulate and hippocampus using the Wake Forest University Pickatlas (Maldjian, Laurienti, Kraft & Burdette, 2003). For clusters that were identified using the ROI approach, correction for multiple comparisons was achieved using a small volume correction (SVC). The diameter applied for small-volume correction was equal to twice the length of the smoothing

kernel (i.e., 24mm) to ensure appropriate spatial resolution. All reported clusters using the SVC were corrected for multiple comparisons using a family-wise error correction of $p_{FWE} < .05$.

Correlations between volume, cortisol and early life stress

Once statistically significant clusters were identified, signal was extracted from SPM and entered into SPSS (SPSS for Windows, Rel. 15.0. 2006. Chicago: SPSS Inc.) for further analysis. All SPSS analyses were conducted on a Dell Dimension workstation (Dell, Round Rock, TX), running Windows XP (Microsoft, Redmond, WA). Separate analyses were used to explore the relationship between decreased volume and the CTQ combined physical/sexual abuse scale and total average cortisol for patients and controls. Partial correlations were used to control for the effects of age and sex within each group.

CHAPTER III

RESULTS

CTQ Results

Four of the 15 control subjects were recruited prior to the inclusion of the CTQ into the study protocol and therefore these data were not available. Additionally, one control subject was a statistical outlier, and was excluded. All patients with MDD completed the questionnaire (Table 2). Patients with unipolar depression had significantly higher scores on the CTQ Emotional Abuse scale ($t_{25} = 3.89$, $p = .001$), the Physical Abuse scale ($t_{25} = 4.27$, $p < .000$), Emotional Neglect Scale ($t_{25} = 4.31$, $p < .000$ and the Physical Neglect Scale ($t_{25} = 3.16$, $p = .006$). The Sexual Abuse Scale was marginally significant after correcting for multiple comparisons ($t_{25} = 25$, $p = .011$). We also compared subjects on a combined physical and sexual abuse scale (CTQ PS Scale). For this combined scale, the patients had significantly higher scores than the controls ($t_{25} = 3.67$, $p = .002$).

Salivary Cortisol Results

Due to insufficient saliva concentrations, accurate cortisol estimates were unavailable for two control subjects and three patients. Additionally, one of the control subjects was an outlier and was excluded from subsequent analysis. Average cortisol levels (the sum of all samples divided by seven) for the patient group were elevated when compared to the control group ($t_{25} = - 2.92$, $p = .007$). However, differences in

cortisol secretion between the patient and control groups were greatest for average morning cortisol (t_{25} = - 3.09, p = .006) (Table 2) (Figure 1).

Table 2. Scores on the CTQ subscales and salivary cortisol levels for patients with MDD and healthy controls

Variable	MDD			Healthy Controls		
	n†	*Mean*	*SD*	*n*†	*Mean*	*SD*
CTQ Emotional Abuse Scale*	18	11.4	6.1	10	5.8	0.8
CTQ Physical Abuse Scale*	18	9.7	4.2	10	5.3	0.7
CTQ Sexual Abuse Scale	18	9.4	6.7	10	5.0	0.0
CTQ Emotional Neglect*	18	12.1	4.3	10	6.8	2.1
CTQ Physical Neglect*	18	8.6	4.1	10	5.2	0.4
CTQ Physical and Sexual Abuse Scale*	18	9.6	5.1	10	5.2	0.3
Salivary Cortisol (morning samples)*	15	10.8	3.6	12	9.7	7.7
Salivary Cortisol (all samples)*	15	7.5	2.1	12	5.6	1.2

** p < .01*
† outliers and missing data have been excluded

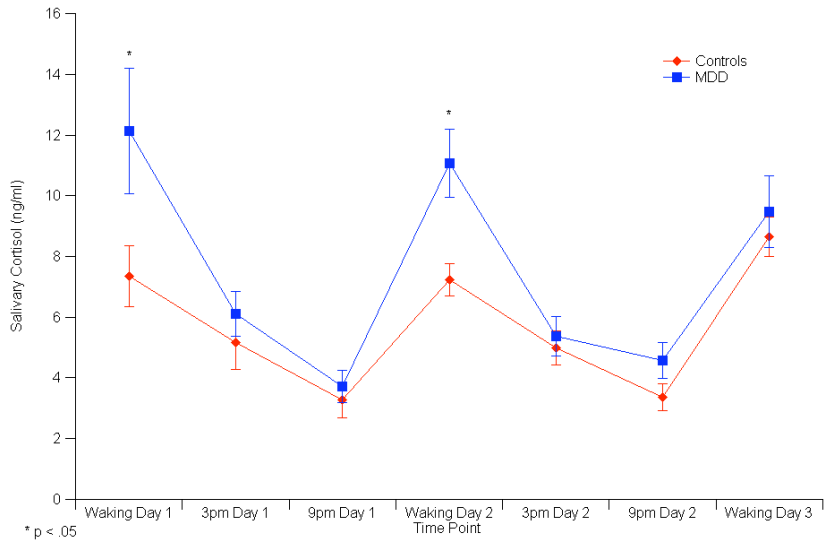

Figure 1. Time course of salivary cortisol (ng/ml) for patients and controls.

Voxel-Based Morphometry Results

Whole brain analysis

There were no significant differences between patients and controls using a whole brain analysis, correcting for multiple comparisons at an alpha set to $p_{FWE} < .05$.

ROI analysis

ROI analysis of volumetric differences between patients and controls revealed an area of decreased volume in the MDD group in the right rostral ACC, BA 32; [x = 17 y = 43 z =2] ($t_{29} = 4.15$, $p_{FWE} = .023$) (Figure 2). No differences between patients and controls were found in the hippocampus at uncorrected thresholds of either p =.001, or p = .01.

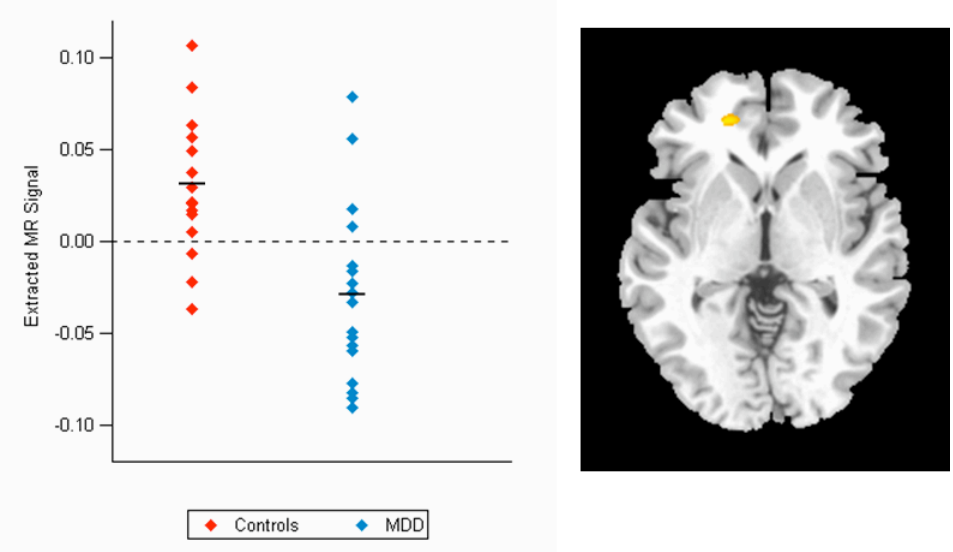

Figure 2. Differences in volume of rACC (Talaraich coordinates [x =17, y= 43, z= 2]) among controls and patients with MDD. Brain image is masked at an uncorrected threshold at p < .01.

Correlations between volume, cortisol and early life stress

Signal was extracted from the maximum voxel value of the right rACC cluster. Within the patient group, individual differences in rACC volume were inversely correlated with both the combined CTQ physical and sexual abuse scales, and average of all cortisol samples. Among the control group, there were no significant correlations between rACC and the CTQ subscales or cortisol (Table 3) (Figure 3).

Table 3. Correlations with peak voxel in rACC (x = 17, y = 43, z = 2), CTQ scores and Salivary Cortisol.†

	df	**r**	**p**
MDD Group			
CTQ physical and sexual abuse - combined*	14	-0.51	0.045
Cortisol (all samples)*	11	-0.60	0.029
Cortisol (morning samples only)	11	-0.25	0.401
Control Group			
CTQ physical and sexual abuse – combined	6	0.41	0.312
Cortisol (all samples)	8	-0.15	0.681
Cortisol (morning samples only)	8	-0.60	0.065

* $p < .05$

†All partial correlations were conducted while controlling for age and sex

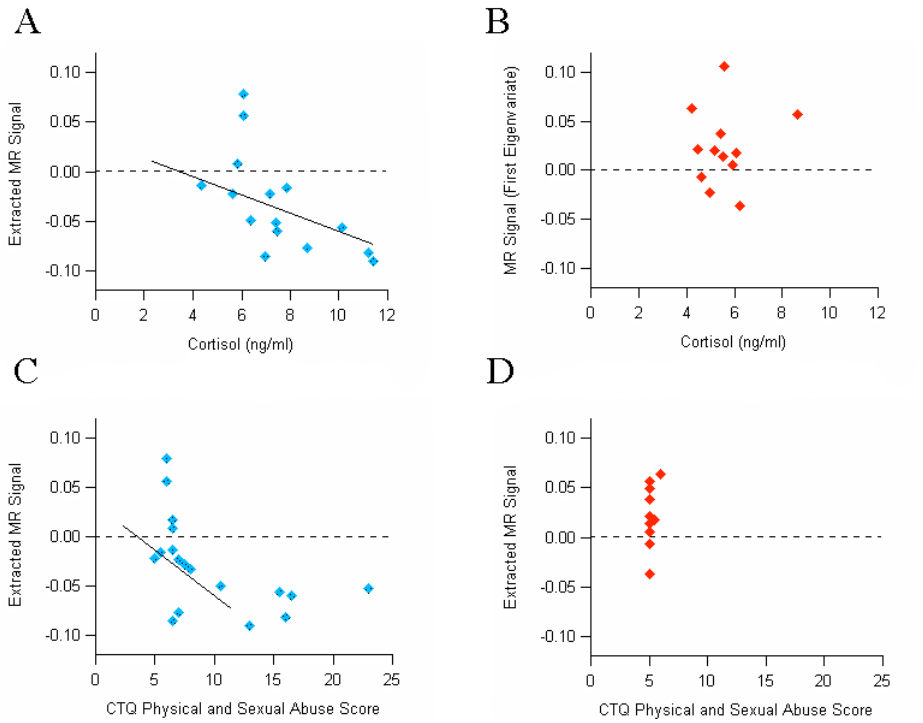

Figure 3. Correlations between volumetric decrease in rACC and average cortisol levels for patients (A) and controls (B) and correlations between volumetric decrease in rACC and the combined CTQ scales for physical and sexual abuse for patients (C) and controls (D).

Post-hoc ROI analyses within the hippocampus

Because prior findings have strongly suggested decreased hippocampal volume associated with MDD, we ran an additional, unplanned analysis in which the CTQ PS subscale used as a covariate in a multiple regression analysis as implemented in SPM2. Within the patient group, we found that CTQ_PS sub-scale was inversely correlated with volume in the right hippocampus [x = 29 y = -36 z = -2] (t_{16} = 4.92, p_{FWE} < .05). However, when signal was extract and age and gender were added as covariates, the correlation was no longer significant (r = -.44, p = .088).

CHAPTER IV

DISCUSSION

The present study found that individuals with MDD had a volumetric decrease in the right rACC (BA 32) as compared to controls. This finding is supported in part by previous studies, where volumes of other sub-regions of the ACC were found to be reduced in individuals with MDD (Hastings et al., 2004; Caetano et al., 2006; Botteron et al., 2002). The study also demonstrated a relationship between decreased rACC volume and both salivary cortisol and a history of early adverse events. These data extend findings from preclinical studies suggesting that observed volumetric decreases in the ACC may result from prolonged exposure to glucocorticoids resulting from chronic stress (Radley et al., 2004; Cerqueira et al., 2005).

Localization of the decrease in cingulate volume to rostral subdivision is particularly relevant, given its responsiveness to stressors that do not pose an immediate physical threat but require higher cortical processing of multiple inputs (e.g., fear, novelty) (Paus, 2001). This region has reciprocal connections with dorsal anterior cingulate as well as subgenual cingulate and the amygdala, and has also been implicated in the neurobiology of depression (Mayberg, 2003). Additionally, two fMRI studies using working memory and attention tasks reported that individuals with MDD exhibited increased rACC activity in order to match the same level of performance as control subjects (Wagner, Sinsel, Sobanski, Kohler, Marinou, Mentzel, et al., 2006; Matuso, Glahn, Peluso, Hatch, Monkul, Najt et al., 2007). This suggests that the rACC may be

less efficient in MDD as compared to controls, which may result from altered ACC morphology. Also consistent with this interpretation is the fact that BOLD fMRI signal has been shown to have a negative correlation with volume within a particular region (Casanova, Srikanth, Baer, Laurienti, Burdette, Hayasaka et al., 2007).

The observed relationship between reduced rACC volume and a history of early adverse events is consistent with prior findings in animals and non-clinical human samples that have revealed a relationship between chronic and repeated stress and cingulate structure (Radley et al., 2004; Cerqueira et al., 2005; Cohen et al., 2006). The correlation between elevated cortisol levels and reduced rACC volume among depressed individuals is also consistent with results from animal studies regarding the role of mPFC in HPA axis negative feedback regulation. It is noteworthy that only the average of all seven samples was correlated with rACC volume, while the average of the morning samples was not. This suggests that volumetric decreases in the rACC are not specifically linked to peak cortisol activity; rather, rACC volume appears to be more closely related to sustained glucocorticoid exposure as determined by the average of the seven samples taken across two and half days.

Our findings suggest early adverse events may act as repeated stressors and serve to initiate glucocorticoid-related injury to the ACC. This may subsequently affect cortico-limbic circuits involved in emotion regulation, as well as negative feedback regulation of HPA activity, potentially playing a role in both the onset of depression and poor regulation of stress. Further research will be required to clarify the temporal relationships between early adverse events, increased HPA activity and structural integrity of the ACC.

We also reported a negative correlation between right hippocampal volume and the CTQ PS scale within the patient group, although there was no main effect of group within this region. However, when MRI signal was extracted and the analysis was conducted while controlling for age and gender, the results were no longer significant ($p = .088$). Therefore, caution should be used in interpreting this result.

The lack of group differences within the hippocampus may result from heterogeneity within our sample of important clinical variables, including the number of episodes, duration of illness and severity of early life trauma. Prior studies that have identified hippocampal decreases associated with MDD have often reported that the extent of hippocampal damage is associated with the duration of illness (Sheline et al., 1999) particularly when it is untreated (Sheline et al., 2001) (cf Campbell and MacQueen, 2006 for a review). In contrast, the number of previous episodes in our sample ranged from none to four or more. Similarly, Vythilingam et al found volumetric decreases in individuals with both MDD and a history of severe child abuse, but not MDD alone (Vythilingam et al., 2002), while the severity of early adverse events in our sample of patients with MDD varied from none to severe. This may explain why we observed a sub-threshold correlation between reported early adverse events and hippocampal volume within the patient group, but did not detect any group differences.

Limitations

Several limitations in the present study warrant mention. First, we did not find any significant differences using a whole-brain analysis after correcting for multiple comparisons, suggesting that where volumetric differences occurred in our depressed

subjects, the effects sizes were only small to moderate. An additional limitation is the use of the VBM method, which is susceptible to normalization and segmentation errors. Patients with MDD were asked to evaluate their history of traumatic childhood experiences while they were in the acute phase of depression, which may have influenced their memory for events. However, it is unlikely that this would explain the observed relationship between rACC volume and reported early adverse events since rACC is not known to serve a functional role in long-term memory. Our study was limited by its reliance on salivary cortisol as the only measure of HPA activity, as opposed to other forms of assessment of HPA function such as the dexamethasone suppression test or the corticotrophin releasing hormone (CRH) test. Finally, the complete neurobiological mechanisms by which elevated cortisol precipitates structural damage in the ACC are likely to involve additional variables that were not evaluated in the present study.

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