Citation for published version (APA):
The onset of psychosis is thought to involve interactions between environmental stressors and the brain, with cortisol as a putative mediator. We examined the relationship between the cortisol stress response and brain structure in subjects at ultra-high risk (UHR) for psychosis. Waking salivary cortisol was measured in 22 individuals at UHR for psychosis and 17 healthy controls. Grey matter volume was assessed using magnetic resonance imaging at 3 T. The relationship between the stress response and grey matter volume was investigated using voxel-based analyses. Our predictions of the topography of cortisol action as a structural brain modulator were informed by measures of brain glucocorticoid and mineralcorticoid receptor distribution obtained from the multimodal neuroanatomical and genetic Allen Brain Atlas. Across all subjects, reduced responsivity of the hypothalamic–pituitary–adrenal (HPA) axis was correlated with smaller grey matter volumes in the frontal, parietal and temporal cortex and in the hippocampus. This relationship was particularly marked in the UHR subjects in the right prefrontal, left parahippocampal/fusiform and parietal cortices. The subgroup that subsequently developed psychosis showed a significant blunting of HPA stress response, observed at trend level also in the whole UHR sample. Altered responses to stress in people at high risk of psychosis are related to reductions in grey matter volume in areas implicated in the vulnerability to psychotic disorders. These areas may represent the neural components of a stress vulnerability model.

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INTRODUCTION

The onset of psychosis is thought to involve interactions between psychosocial stressors in the environment and genetic factors that alter the brain such that there is an increased vulnerability to psychosis. The effects of environmental stressors on the brain are thought to be mediated by the hypothalamus–pituitary–adrenal (HPA) axis, which responds to stress by releasing cortisol into the bloodstream.1 Cortisol interacts with glucocorticoid (GRs) and mineralcorticoid (MRs) receptors which function as transcriptional regulators, but also modulate the responsiveness of the HPA axis via feedback inhibition of corticotropin-releasing hormone and adrenocorticotropic hormone release, such that homeostasis is re-established once stressors abate.2 The repeated or chronic exposure to stress leads to hyperactivity of the HPA axis, resulting in elevated basal cortisol levels and impaired responsiveness to further stress.

According to the neural diathesis-stress model of psychosis, the HPA axis mediates the relationship between exposure to stressors and the emergence of psychotic symptoms, with the suggestion that elevated cortisol levels augment dopamine synthesis.3 This model is supported by evidence that patients with a psychotic disorder have increased circulating levels of cortisol4 and a blunted cortisol response to stress,5 either in the form of experimental psychosocial stressors or the minor physiological stressor of awakening.6 The blunted cortisol response to stress is thought to reflect the impaired responsiveness of a desensitized system.7 Similar findings have recently been reported in individuals at ultra-high risk (UHR) of developing psychosis.8–11

GRs and MRs are both expressed in the brain where corticosteroid hormones act as transcription factors and regulate gene expression.1 Data from animals and humans suggest that the HPA-axis stress-induced dysregulation and the consequent increased release of corticosteroids is associated with an enduring effect on brain structure, with the highest impact on areas undergoing developmental changes at the time of the insult.2 Thus, chronic corticosteroid exposure in rodents, both due to experimental administration or chronic stress, is associated with a reduction in dendritic branching in hippocampal and prefrontal regions.12,13 Similarly, studies in humans exposed to stress or hypercortisolemia show reductions in hippocampal14 and prefrontal volume.15

There have been remarkably few studies of the relationship between alterations in HPA axis function and neuroimaging abnormalities in psychosis. An inverse correlation between hippocampal volume and cortisol levels has been observed in patients with first episode psychosis,7 although interpretation of
this finding is complicated by the possible effects of illness or medication on both variables. These potentially confounding factors can be overcome by studying individuals at UHR for the disorder, who are usually medication-naive. UHR subjects show extensive alterations in grey matter volume irrespective of whether they subsequently develop the disorder, suggesting that these represent neural correlates of their vulnerability to psychosis. The only previous study in this group did not find a significant relationship between cortisol levels and either hippocampal or pituitary volume. However, the study used a regions of interest approach; therefore, the rest of the brain was not examined. Cortisol levels were assessed at a single time point via a blood sample. Serial samples provide a better index of HPA function, and the findings from blood samples can be confounded by the stress associated with venipuncture.

In the present study, we examined the relationship between the cortisol response on waking and whole-brain grey matter volume in UHR individuals. Cortisol was measured in serial salivary samples, and magnetic resonance imaging data were acquired on a 3 T scanner. We used the information on the regional expression of GR and MR in the brain to inform our predictions of the areas most likely to be related to HPA axis responsivity. On the basis of previous findings, we expected that UHR subjects would show a blunted waking cortisol response compared with controls. We then tested the hypothesis that there would be a significant relationship between blunted cortisol response and grey matter volume reductions in the hippocampus and the prefrontal cortex. A subsidiary hypothesis was that this relationship would be more pronounced in the UHR individuals than in controls.

**MATERIALS AND METHODS**

**Ethical approval**

The study was approved by the joint South London and Maudsley National Health Service Foundation Trust Ethics Committee and all participants gave written consent to participate after full details of the study were explained.

**Participants**

Twenty-six individuals meeting criteria for an at-risk mental state (ARMS) were recruited from OASIS (Outreach and Support in South London), a clinic for people at risk of developing psychosis within the same sociodemographic area. Participants were aged 18 to 30 years and were excluded if their intelligence quotient was below 70, if they had a family history of a neurological disorder or severe head injury or if they met DSM-IV criteria for an alcohol or substance dependence disorder other than nicotine. An additional exclusion criterion for control subjects was a family history of psychosis. All the UHR participants were followed up by OASIS for at least 2 years after first contact and monitored for signs of transition to psychosis.

**Clinical measures**

CAARMS-positive and Negative Syndrome Scale (PANSS), Hamilton Anxiety Rating Scale (HAM-A) and Hamilton Depression Rating Scale (HAM-D) were used on the day of scanning to assess and rate symptom severity. Participants were instructed to wake up before 1000 h to collect saliva samples immediately at awakening (0 minutes) and then after 30 and 60 minutes. They were asked to abstain from consuming alcohol the night preceding collection and asked not to eat, drink, brush their teeth or engage in physical activity during the 60-minute collection period. Samples were stored at a temperature of ~20 °C until they were centrifuged at 3500 rpm for 10 minutes at 6 °C to separate saliva from the plasma. It was then transferred from the Salivettes to microtubes and stored at ~80 °C until a continuous, automated, competitive chemiluminescence immunoassay was performed using the Immulite immunoassay analyzer system (DPC; www.diagnostics.siemens.com) to determine free cortisol concentration. The percentage cross-reactivity of the antiserum with cortisone and prednisolone was 0.35% and 27.5%, respectively. The area under the curve for the cortisol awakening response (CAR) was calculated using cortisol levels at 0, 30 and 60 minutes after awakening with formulae described by Pruessner et al. The validity of the sampling is dependent on timing, with delayed collection leading to an underestimate of peak response.

**Image acquisition and analyses**

Volumetric magnetic resonance images were acquired using a General Electric (Milwaukee, WI, USA) 3 T magnetic resonance system. A whole-brain three-dimensional coronal inversion recovery prepared spoiled gradient echo scan was acquired with echo time 2.82 ms, repetition time 6.96 ms, inversion time 450 ms and flip angle 20º. Group-related differences in grey matter volume (GMV) were analysed using voxel-based morphometry, implemented in SPM8 software (http://www.fil.ion.ucl.ac.uk/spm) running under Matlab 7.4 (MatWorks, Natick, MA, USA). T1-weighted volumetric images were preprocessed using the Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL) SPM8 toolbox, iteratively registering grey matter by nonlinear warping to a template generated using DARTEL to obtain a high-dimensional normalization. A homogeneity check across the sample was followed by smoothing with an 8-mm full-width at half maximum (FWHM) Gaussian kernel. The normalization protocol included a ‘modulatory step’ to correct for the assumption about the absolute grey matter values. We then looked for grey matter voxels in the normalized modulated smoothed data that correlated with CAR in all subjects. Age, gender and antipsychotic medication were modelled in the analysis to reduce the potential impact of these variables on the findings. To identify specific changes not confounded by global volumetric differences, the proportional scaling option was used. We also looked for any existing differences in the relationship between cortisol response and cortical grey matter between UHR participants and controls. We thus used the general linear model to look for brain voxels in which this correlation differed according to the clinical status of the participants (UHR/control).

**Use of a priori biological information to guide statistical inferences**

Neuroimaging studies usually involve the analysis of multiple univariate comparisons, posing a multiple-comparison problem. We here corrected our results based on the expression of corticoid receptors in the brain, using this to threshold our results. Our rationale was that regions that had high levels of these receptors were more likely to be influenced by cortisol, reducing the likelihood that a correlation with local grey matter volume would be a false positive. We used data from the Allen Brain Atlas, a multimodal atlas integrating neuroanatomical and gene expression information in humans. Briefly, the Atlas is based on tissue samples collected postmortem from anatomically diverse regions of six healthy adult human brains. Microarray analyses of the samples gave information on the expression levels of a large number of genes in each of the regions sampled. The information on gene expression distribution across different regions of the brain was then used to build a whole-brain atlas.
defined regions following a widely used template.\textsuperscript{31} Where one of the template regions included more than one sampling site, microarray information from the multiple samples was averaged. In addition, the Brain Allen project designed their microarray analysis such that more than one probe would target the expression of a specific gene. In this case, the expression levels of the GR and MR genes were inferred to be the average expression of the different probes targeting them. We took the mean across subjects, and on the basis that one-third of regulated genes are responsive to both receptor types,\textsuperscript{32} we averaged measures for both GR and MR expression in one brain mask (d) used to flexibly threshold our results according to the expression of cortisol-binding receptors. (a) Information about expression levels of glucocorticoid (GR) and mineralocorticoid (MR) receptors was obtained from several parts of the brain of six healthy adults from the Allen Brain Atlas. (b) Samples obtained from the same region of interest of the template used were averaged. (c) Expression rates of probes targeting the same gene (MR or GR) were averaged. (d) Brain mask ranking regions according to their average expression of glucocorticoid and mineralocorticoid receptors in the healthy brain. The brain is shown in radiological convention (where the left side of the figure is the right side of the brain).

RESULTS

Demographic and clinical characteristics of the sample

26 individuals at UHR for psychosis and 17 healthy controls were originally included. Four UHR subjects had to be excluded due to the poor quality of the cortisol sampling, leaving 22 subjects with data for analysis.

Control and UHR individuals did not differ in terms of age (UHR mean [SD] = 22.45 [4.08] years, controls mean [SD] = 24.24 [4.21] years, \( df = 37, t = -1.33, P = 0.19 \)) or gender (UHR females \( n = 9 \), control females \( n = 7 \), \( P = 0.98 \)). There was a trend for higher estimated premorbid intelligence in control participants (UHR mean [SD] = 110.27 [10.46], controls mean [SD] = 115.45 [7.04], \( df = 37, t = -1.85, P = 0.073 \)).

As would be expected, UHR subjects had higher levels of psychopathology than controls as measured using the CAARMS and the PANSS and lower levels of functioning measured using the Global Assessment of Functioning (GAF). In addition, they showed higher levels of anxiety and depression symptoms as measured using the HAM-A and HAM-D (Supplementary Table 1).

The UHR participants were followed up for at least 2 years after the baseline assessments. Within that period, four subjects (18.2%) developed a psychotic disorder.

Cortisol awakening response

UHR participants showed lower levels of cortisol in response to awakening than controls, although this difference did not reach statistical significance (UHR mean [SD] = 223.84 [233.52] nmol min/l, controls mean [SD] = 320.97 [253.85] nmol min/l, \( df = 37, t = -1.24, P = 0.22 \)). Visual inspection of the data (Figure 2) led to the identification of an outlier in the UHR group, confirmed by computing standard scores (\( z = 3.02 \)). After this subject was excluded, there was a strong trend for a between-group difference (UHR mean [SD] = 190.29 [176.77] nmol min/l, controls mean [SD] = 320.97 [253.85] nmol min/l, \( df = 36, t = -1.87, P = 0.07 \)). The four subjects that subsequently transitioned to psychosis had CAR values significantly lower than controls (UHR-transition mean [SD] = 24.75 [49.50] nmol min/l, controls mean [SD] = 320.97 [253.85] nmol min/l, \( df = 19, t = -2.281, P = 0.034 \)).
cortex, bilaterally. Correlations were also evident in the right hippocampus, the right middle frontal, supramarginal, middle temporal and cingulate gyri, and in the left inferior temporal gyrus, superior parietal cortex and operculum (Figure 3). In all these regions, a blunted cortisol response was associated with smaller grey matter volume.

The correlation between CAR and GMV was significantly stronger in UHR individuals than in controls in the right middle frontal gyrus, the right superior parietal gyrus, the right parietal operculum, the right postcentral gyrus, the left angular gyrus, the left precuneus and the left parahippocampal/fusiform gyrus (Figure 4). Conversely, controls showed a stronger relationship in the left fusiform gyrus (Supplementary Figure 1).

DISCUSSION
This study examined the relationship between grey matter volume in individuals at UHR for psychosis and HPA axis abnormalities. Consistent with a previous finding in a larger sample, there was a trend for a blunting of the CAR in the UHR group. Our first major finding was that there was a significant positive relationship between CAR and regional GMV across all the subjects in bilateral frontal, parietal, and temporal cortices, and the right hippocampus, confirming our initial hypothesis. Consistent with our second hypothesis, this relationship was particularly marked in the UHR group, with impaired responsivity of the HPA axis linked to smaller GMV in the right prefrontal, left parahippocampal/fusiform and parietal cortices. The findings in the prefrontal and parahippocampal cortex are of particular interest, as these are the two brain regions most consistently implicated in animal models of psychosis, and patients with psychosis. The group differences at the neuroimaging level may have been more significant than those in the cortisol responses because they provide a more direct measure of the underlying pathophysiology.

Attenuated cortisol responses to stress in UHR individuals are thought to reflect a desensitization of the HPA axis that may increase the vulnerability to psychosis. It has also been suggested that HPA axis abnormalities may alter normal brain maturational processes. Exposure to stress during key periods of vulnerability may slow brain development as corticosteroids influence neurogenesis and neuroplasticity, affecting levels of neurotrophins such as BDNF. Elevated corticosteroid levels can also be neurotoxic, inducing regression of dendritic processes and decreasing neuronal survival following insults, thereby contributing to neuronal death. These effects could manifest as reductions in regional brain matter volume and could contribute to the emergence of psychotic symptoms.

The hippocampus and prefrontal cortex have been found to be particularly susceptible to the effects of chronic or repeated exposure to stress in both animals and humans. The hippocampus is most susceptible during the first years of life, when it is completing key maturational processes, while the prefrontal cortex remains vulnerable throughout the post-pubertal maturational period that coincides with the peak window of psychosis risk. Alterations in hippocampal volume may therefore...
be a correlate of early exposure to stress and may contribute to sensitization to stress due to the role of the hippocampus in the feedback control of the HPA axis. Desensitization of the HPA axis, mirrored by a heightened perception of daily experiences as stressful may contribute to further abnormalities through the effect of cortisol on brain regions undergoing neurodevelopment later in life, even in the absence of further trauma.


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Supplementary Information accompanies the paper on the Translational Psychiatry website (http://www.nature.com/tp)