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Variation in the Corticotropin-Releasing Hormone Receptor 1 (*CRHR1*) Gene Influences fMRI Signal Responses during Emotional Stimulus Processing

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The corticotropin-releasing hormone (CRH) system coordinates neuroendocrine and behavioral responses to stress and has been implicated in the development of major depressive disorder (MDD). Recent reports suggest that GG-homozygous individuals of a single nucleotide polymorphism (rs110402) in the CRH receptor 1 (*CRHR1*) gene show behavioral and neuroendocrine evidence of stress vulnerability. The present study explores whether those observations extend to the neuronal processing of emotional stimuli in humans. *CRHR1* was genotyped in 83 controls and a preliminary sample of 16 unmedicated patients with MDD who completed a functional magnetic resonance imaging scan while viewing blocks of positive, negative, and neutral words. In addition, potential mediating factors such as early life stress, sex, personality traits, and negative memory bias were examined. Robust differences in blood oxygenation level-dependent (BOLD) signal were found in healthy controls (A allele carriers > GG-homozygotes) in the right middle temporal/angular gyrus while subjects were viewing negative versus neutral words. Among GG-homozygotes, BOLD signal in the subgenual cingulate was greater in MDD participants ($n = 9$) compared with controls ($n = 33$). Conversely, among A-carriers, BOLD signal was smaller in MDD ($n = 7$) compared with controls ($n = 50$) in the hypothalamus, bilateral amygdala, and left nucleus accumbens. Early life stress, personality traits, and levels of negative memory bias were associated with brain activity depending on genotype. Results from healthy controls and a preliminary sample of MDD participants show that *CRHR1* single nucleotide polymorphism rs110402 moderates neural responses to emotional stimuli, suggesting a potential mechanism of vulnerability for the development of MDD.

Introduction

The corticotropin-releasing hormone (CRH) system plays a critical role in coordinating the autonomic, endocrine, and behavioral responses to stress (Dunn and Berridge, 1990; Owens and Nemeroff, 1991). Dysregulated CRH systems have been associated with major depressive disorder (MDD) (Arborelius et al., 1999). Hypophyseal CRH hypersecretion may cause ACTH and cortisol dysregulation in MDD (Holsboer and Barden, 1996), and central CRH hypersecretion has been demonstrated by elevated basal levels of CRH-like immunoreactivity in the CSF of MDD patients and suicide victims (Nemeroff et al., 1984; Bánki et al., 1987; Widerlöv et al., 1988; Arató et al., 1989). One consequence of CRH hypersecretion may be the downregulation of its major receptor, CRHR1. Postmortem studies have shown that individuals with MDD have decreased CRH binding and levels of *CRHR1* mRNA in the frontal cortex, suggesting the role for CRHR1 in the pathophysiology of MDD (Nemeroff et al., 1988; Merali et al., 2004).

Several recent studies have suggested that single nucleotide polymorphisms (SNPs) in the *CRHR1* gene are associated with increased incidence of MDD (Liu et al., 2006), and predict antidepressant treatment response (Licinio et al., 2004; Liu et al., 2007). Furthermore, *CRHR1* polymorphisms interact with stressful life experiences to predict MDD (Bradley et al., 2008; Wasserman et al., 2008; Heim et al., 2009; Polanczyk et al., 2009; Wasserman et al., 2009; Grabe et al., 2010) and suicide (Wasserman et al., 2008), as well as the initiation and heavy use of alcohol (Blomeyer et al., 2008; Schmid et al., 2010).

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In particular, the common intronic *CRHR1* SNP rs110402 has been shown to be an important moderator of childhood abuse on hypothalamic-pituitary-adrenal axis functioning and development of MDD (Bradley et al., 2008; Heim et al., 2009; Polanczyk et al., 2009; Tyrka et al., 2009; Ressler et al., 2010). The rs110402 A allele has been shown to have a protective effect against MDD in individuals exposed to childhood abuse (Bradley et al., 2008; Polanczyk et al., 2009). However, beyond neuroendocrine effects, it is not known whether *CRHR1* rs110402 affects brain regions involved in emotion processing and the pathophysiology of MDD.

The present study uses functional magnetic imaging (fMRI) in healthy controls and a preliminary sample of MDD patients to examine how rs110402 genotypes influence brain responses to emotional stimuli. Four *a priori* brain regions previously shown to exhibit abnormal activity in MDD were examined: the subgenual cingulate cortex, hypothalamus, amygdala, and nucleus accumbens (Drevets, 1999; Whalen et al., 2002; Bao et al., 2008; Pizzagalli et al., 2009). As suggested by the studies above, early life stress as well as sex (Heim et al., 2009) were examined as potential factors mediating the effects of rs110402 on brain activity. Furthermore, vulnerable personality traits were hypothesized to be associated with the effects of rs110402, including the Neuroticism dimension of the Revised NEO Personality Inventory (Costa and McCrae, 1992), and the Behavioral Inhibition System scale, which measures aversive motivation relating to feelings of fear, anxiety, frustration, and sadness (Gray, 1990; Carver and White, 1994).

Materials and Methods

Subjects. Genotyping was performed in 128 healthy controls (70 males, mean age ± SD, 26 ± 6 years; 58 females, 28 ± 8 years), and 22 unmedicated patients with current MDD (8 males, 41 ± 12 years; 14 females, 39 ± 11 years). Controls were screened for active medical illness and for current or past psychiatric disorders in themselves or first-degree relatives. MDD volunteers were diagnosed with the Structured Clinical Interview for DSM-Clinical Version (SCID-IV), scored >15 (mean score ± SD, 19 ± 3) on the 17-item Hamilton Depression Rating Scale, and were free of antidepressant medication for at least 6 months at the time of the study (mean duration of illness ± SD, 21 ± 32 months; range, 1.5–132 months). None of the volunteers were taking psychotropic medications, including hormones or hormonal contraception in women.

Ninety-nine controls and 22 MDD were asked to complete an fMRI emotion word stimulus task. Pregnancy tests and urine drug screens were confirmed negative before scanning. Two patients with MDD did not complete the task, and 16 controls and four MDD patients were excluded for artifacts in fMRI data or excessive head movement (>2 mm of translation or 2 degrees of rotation) that resulted in less than two valid scan sessions. In total, usable fMRI data were obtained from 83 controls (45 males, mean age ± SD, 27 ± 7 years; 38 females, 29 ± 8 years) and 16 patients with MDD (5 males, 43 ± 13 years; 11 females, 39 ± 12 years).

Written informed consent was obtained and study protocols were approved by the Institutional Review Board of the University of Michigan Medical School.

Genotyping. DNA was extracted from whole blood. A genomic region of chromosome 17 containing sequence 5 kb upstream and 1 kb downstream of *CRHR1* was retrieved from NCBI Human Build 35.1. *CRHR1* rs110402, located in intron 2, was genotyped using the Illumina GoldenGate platform (Hodgkinson et al., 2008). The genotyping in controls (AA = 27, AG = 54, GG = 47) was in Hardy Weinberg Equilibrium ($p = 0.15$). The minor (A) allele frequency was 0.42. Genotypes in the MDD sample were as follows: AA = 6, AG = 4, GG = 12.

To determine whether population stratification might have an influence on the outcome, samples were genotyped for 186 ancestry informative markers (AIMs) (Hodgkinson et al., 2008). The same AIMs were genotyped in 1051 individuals from the 51 worldwide populations represented in the HGDP-CEPH Human Genome Diversity Cell Line Panel (http://www.cephb.fr/HGDP-CEPH-Panel). Structure 2.2 (http://pritch.bsd.uchicago.edu/software.html) was run simultaneously using the AIMs genotypes from our sample and the 51 CEPH populations to identify population substructure and compute individual ethnic factor scores. This ancestry assessment identifies seven ethnic factors (Hodgkinson et al., 2008). Mann–Whitney *U* tests were conducted to compare ethnic factors between A-allele carriers versus GG-homozygous groups, and Spearman's rank correlation coefficients were calculated for ethnic factors versus fMRI BOLD signal.

Emotion word stimulus task. During fMRI, blocks of positive, negative, and neutral words were presented with an LCD video display in the bore of the MR scanner. Subjects were instructed to read the word silently and press a button on a fiber-optic keypad device with their right index finger to indicate that they understood the word. Words were selected from the Affective Norms for English Words (ANEW) list, which provides a normative emotional rating for a large number of words in the English language (Bradley and Lang, 1999). The words in the ANEW list were rated on the dimensions of valence and arousal on a scale of 1 (negative valence; low arousal) to 9 (positive valence; high arousal). For the present study, we chose negative words from this list that had an average valence rating of <3, neutral words with valence ratings between 4.5 and 5.5 and positive words with a valence rating >7. Standard deviations for all valence ratings were <2 and arousal ratings were >3. Word length ranged from 3 to 11 letters (mean number of letters ± SD, 6.04 ± 1.64).

Words were presented one at a time (3 s, followed by 1 s cross-hair orientation) in blocks of six words of the same valence. Six blocks (with two blocks from each condition: positive, negative, neutral) constituted one run. Nonactive rest blocks of 18 s were interspersed between each block. Block order was counterbalanced using a Latin squares design in each of three runs. Subjects with usable data from at least two runs were included in the analyses.

Following the scan, a subset of control ($n = 18$) and MDD ($n = 18$) subjects were administered a memory recall and recognition test without prior warning to evaluate implicit recall and recognition memory, using percentage word recall and recognition, respectively. Negative memory bias is characteristic of MDD (Leppänen, 2006) and was determined by subtracting percentage recall or recognition of neutral words from percentage recall or recognition of negative words. These values were used for group comparisons and regression with fMRI activity.

The emotional words task was chosen over other emotional probes (e.g., faces, pictures) to test the hypothesis that cognitive distortions are affected by vulnerable genotypes. Emotional words may require more cognitive processing effort (i.e., memory, associations), which may be more subtly influenced by functional differences in *CRHR1*. For example, recent evidence suggests that *CRHR1* modulates early-life stress-mediated cognitive deficits in animals (Ivy et al., 2010; Wang et al., 2011).

fMRI data acquisition. Whole-brain scans were performed using a 3.0 tesla Signa scanner (GE Healthcare) using a standard radio frequency coil. Blood oxygenation level-dependent (BOLD) signal was acquired using a T2*-weighted pulse sequence (repetition time, 2000 ms; echo time, 30 ms; flip angle, 90°; field of view, 24 cm, 64 × 64 matrix; 1 voxel, 3.75 × 3.75 × 4 mm) with single-shot combined spiral in/out acquisition (Glover and Law, 2001), which has been shown to reduce signal dropout in areas prone to high susceptibility artifacts. The entire volume of brain (30 slices) was acquired at each repetition time. A high resolution T1-weighted pulse sequence was acquired to provide anatomical localization (three-dimensional spoiled gradient recalled echo; repetition time, 24 ms; echo time, 5 ms; flip angle, 45°; field of view, 24 cm, 256 × 256 matrix; slice thickness, 1.5 mm).

fMRI data analysis. BOLD contrasts were slice-time corrected, realigned, smoothed with an 8 × 8 × 8 Gaussian filter, and analyzed with Statistical Parametric Mapping v.2 (SPM2; Wellcome Institute of Cognitive Neurology, London, UK). Contrast *t* maps for each subject were derived using a primary subtraction of negative or positive word blocks minus neutral word blocks (Neg − Neut or Pos − Neut) with head movement regressors, normalized with linear and nonlinear warping to standard (Montreal Neurological Institute) space and smoothed with a 6 mm Gaussian filter to reduce residual interindividual anatomical vari-

ability. Interindividual, random-effects analyses were performed using SPM2.

Control subjects with the potentially protective *CRHR1* rs110402 A allele were grouped (i.e., AA and AG) to increase power. Whole-brain analysis was conducted for comparing A-carriers versus GG-homozygous individuals in the Neg − Neut and Pos − Neut contrasts. Threshold was set at $p < 0.05$, correcting for false discovery rate (FDR) for whole brain, two-tailed. Peak activation ($p < 0.025$, FDR-corrected) was extracted for further analyses using MarsBaR VOI toolbox (version 0.38) (Brett et al., 2002) for SPM and analyzed with SPSS statistical software (version 16.0) to plot the data and rule out the presence of outliers using the Tukey box plot, where an outlier is defined as a score >1.5 interquartile lengths from the first or third quartile.

For comparing MDD patients with controls, volumes of interest (VOI) analyses were chosen over whole-brain analyses because of the small sample size of MDD patients. Four *a priori* brain regions previously shown to exhibit abnormal activity in MDD were examined: the subgenual cingulate cortex (Drevets et al., 1999; Mayberg et al., 1999, 2000; Langenecker et al., 2007; Keedwell et al., 2010), hypothalamus (Bao et al., 2008), bilateral amygdala (Whalen et al., 2002), and bilateral nucleus accumbens (Pizzagalli et al., 2009). These regions are also closely interconnected (Hsu and Price, 2007, 2009) and have high levels of CRHR1 (Millan et al., 1986; Sánchez et al., 1999). Activation in these VOIs was considered statistically significant at $p < 0.05$, FDR-corrected within VOI, two-tailed. Using anatomical boundaries, VOIs were manually created for the subgenual cingulate cortex (rectangular box), hypothalamus (rectangular box), and nucleus accumbens (sphere); MarsBaR automated anatomical labeling (Tzourio-Mazoyer et al., 2002) was used for the amygdala (Table 1). Alpha levels were Bonferroni-adjusted for four comparisons ($p = 0.0125$).

Questionnaires. Vulnerable personality traits examined included the neuroticism dimension of the Revised NEO Personality Inventory (PI) (Costa and McCrae, 1992) and the Behavioral Inhibition System (BIS) scale, which measures aversive motivation relating to feelings of fear, anxiety, frustration, and sadness (Gray, 1990; Carver and White, 1994). Correlations were considered statistically significant at $p < 0.05$, two-tailed. Correlations with the six facets of neuroticism (anxiety, hostility, depression, self-consciousness, impulsiveness, vulnerability to stress) were considered statistically significant after Bonferroni-adjusting for six comparisons.

Early life stress was scored with the Childhood Trauma Questionnaire (CTQ) (Bernstein et al., 1994) and specific items from the Family Experiences Questionnaire (FEQ) (Durrett et al., 2004). The CTQ is a 25-item inventory that provides a brief, reliable, and valid measure of childhood abuse and neglect. The FEQ is a more comprehensive evaluation of childhood experiences with 133 items. Ten items from the FEQ that overlapped with the CTQ were chosen, including sexual contact, physical abuse, physical neglect, and emotional neglect, each weighted by the length/severity of these episodes. Most control subjects completed the FEQ ($n = 72$) and some completed the CTQ ($n = 11$). Standard (z) scores were computed for total raw scores from the CTQ and FEQ and used for group comparisons and regression with fMRI activity in controls. Thirteen patients with MDD completed the CTQ only.

Results

Whole-brain analysis of Neg − Neut blocks in healthy controls showed greater regional activation in A-carriers ($n = 50$) compared with GG-homozygotes ($n = 33$). Robust differences were found in a cluster that spanned the right middle temporal gyrus [posterior part; Brodmann area (BA) 21] and angular gyrus (BA 39) ($p = 0.011$, FDR-corrected for whole brain, two-tailed; Fig. 1). Extraction of peak differences in the angular gyrus showed greater activation to negative words in A-carriers, and greater activation to neutral words in GG-homozygotes ($t_{81} = 5.61$, $p < 0.025$, FDR-corrected for whole brain, two-tailed). Mean BOLD percentage change values extracted from peak activation for Neg − Neut (±SEM) were as follows: A-carriers, 0.070 (0.017); GG-homozygotes, −0.110 (0.027). No suprathreshold clusters were found for GG > A-carriers in healthy controls. No significant effects were found in Pos − Neut comparisons, indicating the gene effect is specific to negative words and not simply to words with high arousal. Sex and age entered as covariates did not affect these results. There were no differences between MDD patients and healthy controls, stratified by genotype, in a whole-brain analysis.

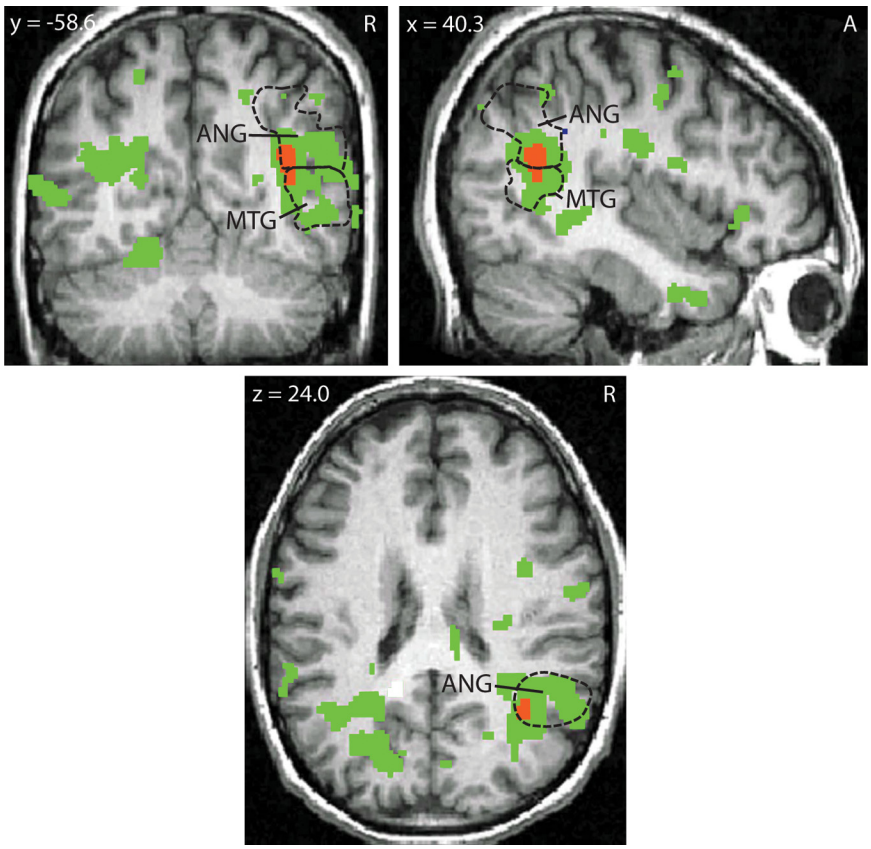

Figure 1. fMRI BOLD activity (Neg − Neut) in controls: A-carriers > GG-homozygotes. Peak activation differences in a whole-brain analysis found in the right middle temporal/angular gyrus. Green areas indicate $p < 0.05$, FDR-corrected for whole brain, two-tailed. Red area indicates $p < 0.025$, FDR-corrected for whole brain, two-tailed, cluster size = 560 mm^3; Montreal Neurological Institute stereotactic space coordinates of peak activation: $x = 38$, $y = -60$, $z = 26$. Dashed lines indicate the anatomical boundaries of the right middle temporal and angular gyrus. ANG, Angular gyrus; MTG, middle temporal gyrus; A, anterior; R, right.

Positive relationships between behavioral traits and BOLD responses were found in GG-homozygotes, but not A-carriers. Activation in the Neg − Neut BOLD contrast in the middle temporal/angular gyrus was significantly correlated with neuroticism ($r = 0.42$, $p = 0.034$) and the neuroticism facet vulnerability to

Table 1. VOI analysis (Neg − Neut) in MDD patients versus controls

VOI	VOI center of mass	VOI volume (mm^3)	Peak activation	*t*
GG-homozygotes				
MDD patients (GG) > controls (GG)				
Subgenual cingulate (BA 25/32)	0, 24, −7	2520	−2, 28, −6	4.60*
MDD patients (GG) > controls (all)				
Subgenual cingulate (BA 25/32)	0, 24, −7	2520	−2, 28, −6	4.10*
A-allele carriers				
Controls (A−) > MDD patients (A−)				
Hypothalamus	0, −5, −11	1080	4, −6, −12	3.96*
Amygdala (L)	−24, −2, −19	1760	−30, 0, −18	3.81
Amygdala (R)	27, −1, −19	1984	26, 0, −20	3.67
Nucleus accumbens (L)	−8, 8, −10	264	−8, 8, −10	3.56*
Nucleus accumbens (R)	8, 8, −10	264	10, 8, −8	2.05
Controls (all) > MDD patients (A−)				
Hypothalamus	0, −5, −11	1080	6, −6, −10	3.46
Amygdala (L)	−24, −2, −19	1760	−30, 2, −18	3.45*
Amygdala (R)	27, −1, −19	1984	26, 0, −20	3.98*
Nucleus accumbens (L)	−8, 8, −10	264	−6, 10, −12	2.76
Nucleus accumbens (R)	8, 8, −10	264	6, 10, −8	1.45

VOI center of mass and Peak activation in *x*, *y*, *z* coordinates from Montreal Neurological Institute stereotactic space. Alpha levels were Bonferroni-adjusted to 0.0125 for examining four brain regions. *$p < 0.01$, FDR-corrected within VOI, two-tailed. R, Right; L, left.

stress ($r = 0.51$, $p = 0.008$), as well as with behavioral inhibition ($r = 0.45$, $p = 0.022$). Controlling for sex in a partial correlation showed that sex explained the relationship between BOLD responses and neuroticism ($r = 0.263$, $p = 0.204$), vulnerability to stress ($r = 0.417$, $p = 0.038$), and behavioral inhibition ($r = 0.284$, $p = 0.170$). Controlling for age did not affect the zero-order correlation. Further analyses revealed that female GG-homozygotes had significantly higher scores than male GG-homozygotes for neuroticism ($t_{24} = 2.42$, $p = 0.023$) and behavioral inhibition ($t_{24} = 2.74$, $p = 0.011$), but not for vulnerability to stress ($t_{24} = 1.78$, $p = 0.088$). Female A-carriers were not significantly different from male A-carriers on these three traits.

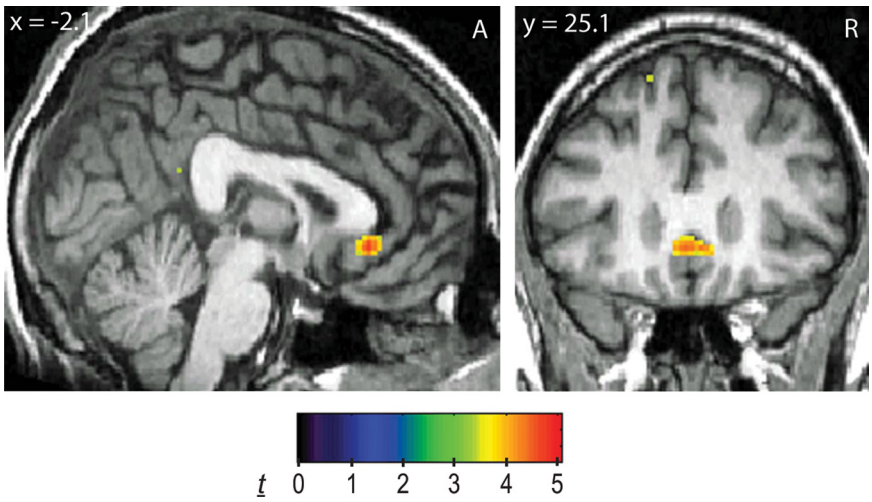

Figure 2. fMRI BOLD activity (Neg − Neut) for GG-homozygotes: MDD patients > controls. Display of whole-brain activation showing specific difference in the subgenual cingulate. Display threshold: $p < 0.001$. A, Anterior; R, right.

Differences between MDD patients and healthy controls, stratified by genotype, were also tested in the four *a priori* brain regions, with alpha levels Bonferroni-adjusted for four comparisons. Among GG-homozygotes, BOLD signal in the subgenual cingulate was greater in MDD patients ($n = 9$) compared with controls ($n = 33$) in response to Neg − Neut words ($t_{40} = 4.60$, $p = 0.0014$, FDR-corrected within VOI, two-tailed; Table 1). Display of whole-brain activation shows specificity in the subgenual cingulate (Fig. 2). Mean BOLD percentage change values extracted from peak activation for Neg − Neut (±SEM) were as follows: controls, −0.063 (0.027); MDD, 0.181 (0.052). Conversely, among A-carriers, BOLD signal was smaller in MDD patients ($n = 7$) compared with controls ($n = 50$) in the hypothalamus ($t_{55} = 3.96$, $p = 0.0037$, FDR-corrected within VOI, two-tailed) and left nucleus accumbens ($t_{55} = 3.56$, $p = 0.0058$, FDR-corrected within VOI, two-tailed; Table 1). Bilateral amygdala activations approached Bonferroni-adjusted alpha levels (left: $t_{55} = 3.81$, $p = 0.015$; right: $t_{55} = 3.67$, $p = 0.015$, FDR-corrected within VOI, two-tailed; Table 1). Display of whole-brain activation shows specificity in these areas (Fig. 3). Mean BOLD percentage change values extracted from peak activation for Neg − Neut (±SEM) were as follows: hypothalamus (Fig. 3*A*): controls, 0.070 (0.024); MDD, −0.197 (0.083); left amygdala (Fig. 3*B*): controls, 0.011 (0.026); MDD, −0.279 (0.068); right amygdala (Fig. 3*B*): controls: −0.008 (0.039); MDD, −0.464 (0.158); left nucleus accumbens (Fig. 3*C*): controls, 0.048 (0.020); MDD, −0.146 (0.052). Similar results were obtained when A-carrier MDD subjects or GG-homozygote MDD patients were compared with all controls, regardless of genotype, including significant activation in the subgenual cingulate and bilateral amygdala (Table 1). No suprathreshold clusters were found for controls (GG-homozygotes or all controls) > MDD or MDD > controls (A-carriers or all controls). Sex and age entered as covariates did not affect these results.

Control analyses indicated that genetic/ethnic stratification did not account for these findings. In our study sample the mean (SD) [median] ethnic factor scores were as follows: European, 0.66 (0.39) [0.90]; African, 0.14 (0.30) [0.00]; Asian, 0.08 (0.18) [0.01]; Mid-Eastern, 0.07 (0.17) [0.02]; Far Eastern, 0.02 (0.11) [0.00]; Oceania, 0.01 (0.02) [0.00]; America, 0.01 (0.04) [0.00]. The seven ethnic

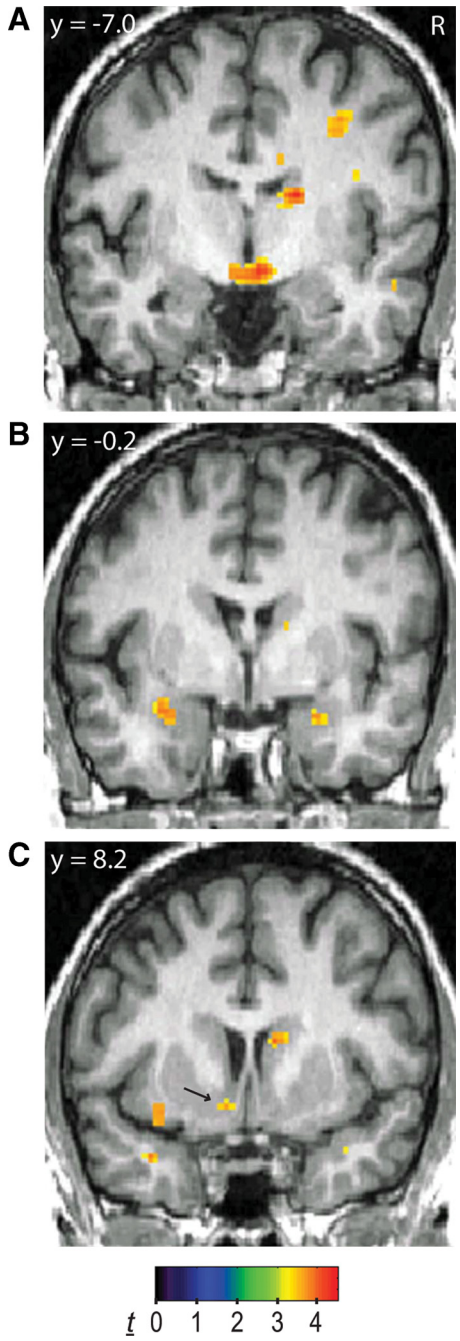

Figure 3. fMRI BOLD activity (Neg − Neut) for A-carriers: controls > MDD patients. ***A–C***, Display of whole-brain activation showing specific differences in the hypothalamus (***A***), bilateral amygdala (***B***), and left nucleus accumbens (***C***, arrow). Display threshold: $p < 0.001$. R, Right.

factors did not differ significantly between A-carriers and GG-homozygotes in controls or MDD patients (alpha level Bonferroni-adjusted to 0.05/7 = 0.007; z, −2.30–2.12; p, 0.02–0.67). Spearman's rank correlation did not reveal significant relationships between ethnic factors and Neg − Neut BOLD percentage change in the angular gyrus in controls (alpha level Bonferroni-adjusted to 0.007; r_s, −0.20–0.07; p, 0.07–0.93). In addition, Spearman's rank correlation did not reveal significant relationships between ethnic factors and Neg − Neut BOLD percentage change in the extracted VOIs (alpha level Bonferroni-adjusted to $0.05/[7 \times 5] = 0.001$) in controls or MDD patients (subgenual cingulate cortex: r_s, −0.17–0.22; p, 0.03–0.82; hypothalamus, r_s, −0.12–0.21; p, 0.04–0.93; left amygdala: r_s, −0.12–0.23; p, 0.02–0.85; right amygdala: r_s, −0.19–0.14; p, 0.06–0.89; left accumbens: r_s, −0.19–0.10; p, 0.07–0.87).

In controls, early life stress scores were not associated with Neg − Neut BOLD activity in the middle temporal/angular gyrus in A-carriers or GG-homozygotes. In VOIs, early life stress scores were negatively correlated with Neg − Neut BOLD activity in the hypothalamus ($r = -0.30$, $p = 0.046$) only in the A-carriers ($n = 45$). Conversely, early life stress scores were negatively correlated with Neg − Neut BOLD activity in the left amygdala ($r = -0.36$, $p = 0.056$) only in the GG-homozygotes ($n = 29$), although this relationship was not statistically significant. Early life stress scores did not differ between A-carriers and GG-homozygotes. In the preliminary sample of MDD patients, total CTQ scores, or specific items such as physical abuse, were not correlated with activity in the brain regions examined.

Negative recognition (but not recall) memory bias was greater in MDD patients compared with controls ($t_{34} = 2.66$, $p = 0.01$, two-tailed). In controls, negative recognition memory bias was negatively correlated with Neg − Neut BOLD activity in the hypothalamus in GG-homozygotes ($r = -0.76$, $p = 0.046$), and positively correlated in A-carriers, although this did not reach significance ($r = 0.58$, $p = 0.080$). In MDD patients, negative recall memory bias was also positively correlated with Neg − Neut BOLD activity in the subgenual cingulate, but only in GG-homozygotes ($r = 0.81$, $p = 0.008$).

Discussion

To our knowledge, this is the first study to examine the effect of *CRHR1* SNP rs110402 on neuronal emotional processing. This SNP, which has been associated with dysregulated neuroendocrine stress responses and MDD, showed robust activity differences in the right middle temporal/angular gyrus between A-carriers and GG-homozygotes in response to emotional lexical stimuli in healthy controls. In a preliminary sample of MDD patients, GG-homozygotes showed greater BOLD response compared with controls in the subgenual cingulate, a region implicated in the pathophysiology of MDD (Drevets et al., 1999; Mayberg et al., 1999, 2000; Langenecker et al., 2007; Keedwell et al., 2010). Conversely, A-carriers with MDD showed lesser BOLD responses compared with controls in the hypothalamus, amygdala, and nucleus accumbens. These findings identify brain regions and activity patterns that may mediate the association between this SNP and vulnerability to MDD.

BOLD response to Neg − Neut words was significantly greater in A-carriers compared with GG-homozygotes in the right middle temporal/angular gyrus (BA 21/39). This difference was driven in part by lesser Neg − Neut activity in GG-homozygotes. Lesser activity in the middle temporal gyrus (BA 21) in response to Neg − Neut words has also been found in a sample of healthy subjects (Kuchinke et al., 2005), although genotype was not examined. It is possible that this lesser activity was driven by GG-homozygotes, since our results suggest that there is overall lesser activity in this region when the two groups are combined. Similarly, cerebral blood flow decreased in the angular gyrus when bank officials were shown videos of an armed robbery that they had recently witnessed (Fredrikson et al., 1997). In a study with MDD patients, Canli et al. (2004) found reduced BOLD activity in response to negative words in the superior temporal gyrus (BA 22) in MDD patients compared with healthy controls. These data, along with the present study, suggest common areas in the temporal cortex specific to semantic retrieval (Price, 2000) and atten-

tional processing of emotional stimuli (Davidson et al., 1999) that exhibit lesser activity in response to negative emotional stimuli. Here we show that this decrease was more pronounced in GG-homozygotes of rs110402.

Greater activity in A-carriers compared with GG-homozygotes in the right middle temporal/angular gyrus might also suggest that A-carriers were more successful at cognitive reappraisal of negative emotions. Using distancing as a method for cognitive reappraisal of negative social scenes, Koenigsberg et al. (2010) found that distancing compared with looking (Neg − Neut) resulted in increased activity in the right middle temporal gyrus, similar to the present comparison of A (Neg − Neut) − GG (Neg − Neut). Similarly, cognitive reappraisal of negative pictures specifically involved the middle temporal and angular gyrus (BA 21/39) (McRae et al., 2010). It is therefore possible that A-carriers in our study were more successful at automatic cognitive reframing; however, the volunteers in our study were not asked to use reappraisal strategies. Consistent with this hypothesis, GG-homozygotes and A-carriers showed differential associations between negative recognition memory bias and Neg − Neut hypothalamic activity. GG-homozygotes showed a negative correlation between negative recognition memory bias, whereas A-carriers showed a positive correlation, although the latter was not statistically significant. This suggests that hypothalamic Neg − Neut activity in A-carriers and GG-homozygotes is differentially mediated by negative memory bias, potentially through different cognitive reappraisal strategies.

Among GG-homozygotes, women showed increased levels of neuroticism and behavioral inhibition compared with men. These sex differences were not found in A-carriers. High neuroticism, as measured by the NEO-PI, is associated with increased risk of MDD (Boyce et al., 1991; Kendler et al., 1993). The BIS and Behavioral Activation System (BAS) are based on a theory of limbic-cortical systems involved in behavioral activation and inhibition in response to punishment or reward (Gray, 1990), and lower BAS/higher BIS levels are found in MDD patients compared with controls (Kasch et al., 2002). Thus, vulnerability to MDD may be increased in female GG-homozygotes. The vulnerability of GG-homozygotes to MDD may in turn depend on exposure to moderate to severe childhood trauma (Bradley et al., 2008; Heim et al., 2009; Polanczyk et al., 2009; Tyrka et al., 2009; Ressler et al., 2010). A significant negative correlation was found between early life stress and hypothalamus activation only in A-carriers, and a near-significant negative correlation was found between early life stress and left amygdala activation only in GG-homozygotes, suggesting that early life stress differentially impacts emotional processing in A-carriers and GG-homozygotes. Larger studies are needed to determine the impact of early life stress on brain activity in A-carriers and GG-homozygotes. For example, one study found a three-way interaction of early abuse, sex, and rs110402 on adult depressive symptoms, whereby the specific types of abuse mediated the effect of sex (Heim et al., 2009).

In our preliminary sample of MDD participants, GG-homozygotes showed greater BOLD activity in the subgenual cingulate compared with controls while viewing Neg − Neut words. This activation was not found in A-carriers, raising the possibility that in MDD, subgenual BOLD activation to negative emotional stimuli is specific to GG-homozygotes of *CRHR1* rs110402. Increased subgenual activation may reflect greater self-referential processing and attempts at emotional regulation in GG-homozygotes, consistent with the hypothesized role of the subgenual cingulate (Berman et al., 2011). Similar to our results, increased subgenual BOLD activity in MDD participants has been observed while viewing sad faces (Gotlib et al., 2005) and during rumination (Cooney et al., 2010) compared with controls. Since the GG genotype has been associated with MDD (Bradley et al., 2008; Polanczyk et al., 2009; Heim et al., 2009; Tyrka et al., 2009; Ressler et al., 2010; present study), it would be of interest to determine whether that effect is more pronounced or even driven by GG-homozygotes, and not A-carriers, with MDD. The possibility that GG-homozygotes of *CRHR1* rs110402 represent a genetically driven subtype of MDD may have clinical implications, as hyperactive subgenual activity has been associated with better treatment response in medication trials (Mayberg et al., 2000). Interestingly, the frequency of GG-homozygotes was greater in MDD patients compared with that of controls (MDD: A- = 10, GG = 12; controls: A- = 81, GG = 47), although a larger sample is needed to conclude that the GG-homozygotes are overrepresented in MDD patients.

A-carriers with MDD showed lesser BOLD activity in the hypothalamus, amygdala, and nucleus accumbens compared with controls while viewing Neg − Neut words. This effect was not found in GG-homozygotes. The A allele of rs110402 has been shown to have a protective effect against MDD in the presence of childhood abuse (Bradley et al., 2008; Heim et al., 2009; Polanczyk et al., 2009). Deactivation in the amygdala and nucleus accumbens may reflect a lack of motivational value for negative stimuli, as both regions are involved in the representation and encoding of stimulus saliency (Zink et al., 2004; Adolphs, 2008). These regions are further interconnected with each other and the hypothalamus (Heimer et al., 1991; Amaral et al., 1992), potentially regulating neuroendocrine function and resulting in a protective effect. Increased and sustained amygdala activity in response to emotional stimuli has been reported in MDD (Sheline et al., 2001; Siegle et al., 2002), however, the decreased amygdala activity in MDD A-carriers found in the present study may reflect a subgroup responding differently to emotional stimuli. The finding that negative recall memory bias was positively correlated to subgenual cingulate activity only in MDD GG-homozygotes suggests differences in the processing of emotional stimuli in A-carriers and GG-homozygotes. How some individuals develop MDD despite having the protective A allele is not known and requires further study.

The mechanism by which rs110402 affects the function of CRHR1 is presently unknown. Variations of rs110402 may affect the regulation of CRHR1 receptors, resulting in the pattern of BOLD activity observed in GG-homozygotes with MDD in the subgenual cingulate. Although CRHR1 regulation has not been specifically studied in the subgenual cingulate, in other prefrontal areas (BA 9, 10, 11), CRH binding sites and *CRHR1* mRNA were downregulated in MDD suicides (Nemeroff et al., 1988; Merali et al., 2004). Limitations of the present study include a relatively small sample of MDD patients, the results from which should be considered preliminary. The observed differences between MDD and controls were relatively large and specific to brain regions with known abnormalities in MDD; however, the small and unbalanced groups limit the generalizability of the results to the MDD population and warrants further investigation and replication in a larger sample before conclusions can be definitively drawn.

In conclusion, the present study demonstrates that *CRHR1* rs110402 influences brain responses to negative emotional stimuli. These include greater Neg − Neut activity in the middle temporal/angular gyrus in A-carriers compared with GG-homozygotes in healthy individuals. In a preliminary sample of MDD patients, GG-homozygotes exhibited greater activity in the subgenual cingulate cortex compared with healthy controls, and

A-carriers exhibited lesser responses in limbic areas compared with healthy controls. The results also show evidence for influencing factors such as early life stress, personality traits, and levels of negative memory bias, that are associated with brain activity to negative emotional stimuli depending on *CRHR1* rs110402. These findings help explain how differences in the *CRHR1* gene may contribute to vulnerability to MDD.

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