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ABSTRACT

Context: The *FKBP5* gene product regulates glucocorticoid receptor (GR) sensitivity and hypothalamic-pituitary-adrenal axis functioning, and has been associated with a number of stress-related psychiatric disorders. The study of intermediate phenotypes, such as emotion-processing biases and their neural substrates, provides a way to clarify the mechanisms by which *FKBP5* dysregulation mediates psychopathology risk.

Objective: To examine whether allelic variations for a putatively functional SNP associated with *FKBP5* gene regulation (rs1360780) would relate differentially to attentional bias for threat; this was measured through behavioral response on a dot probe task and hippocampal activation during task performance. Morphological substrates of differential hippocampal response were also measured.

Design: Cross-sectional study examining associations between genotype, behavioral response and neural response (using fMRI) on the dot probe; Voxel-based morphometry (VBM), global and local shape analyses were used to measure structural differences in hippocampi between genotype groups.

Setting: Participants were recruited from primary care clinics of a publicly-funded hospital in Atlanta, Georgia.

Participants: An African-American cohort (N=103), separated into two groups by genotype: one genotype group included carriers of the rs1360780 'T' allele, which has been previously associated with increased risk for PTSD and affective disorders; the other group did not carry this allele. Behavioral data included both genders (N=103); the MRI cohort (n=36) included only females.

Main Outcome Measures: Behavioral and fMRI (BOLD) response, VBM and shape analyses

Results: Carriers of the rs1360780 'T' allele showed an attention bias towards threat, compared to individuals without this allele. Carriers of this allele demonstrated corresponding increases in

hippocampal activation and differences in morphology; global and local shape analyses revealed alterations in hippocampal shape, for TT/TC, versus CC genotype groups.

Conclusions: Genetic variants of *FKBP5* may be associated with risk for stress-related psychopathology via differential effects on hippocampal structure and function, resulting in altered attentional response to perceived threat.

1. Introduction

In the past decade, *FKBP5* has emerged as a promising genetic candidate for investigations of vulnerability to mood and anxiety disorders[1]. The *FKBP5* gene encodes for the protein FK506 binding protein 5-51, which regulates sensitivity of the glucocorticoid receptor (GR). The GR is a critical component of the body's stress response system and a primary binding site for cortisol, a steroid released during acute and chronic stress. FKBP5 interacts with heat shock protein 90 to reduce GR affinity, modulating cortisol activity and preventing translocation of the GR complex to the nucleus[2]. Through this process, FKBP5 appears to decrease GR sensitivity (or increase GR resistance) to cortisol following stress. Dysregulated GR signaling is common in affective disorders[1, 3] and may be mediated by *FKBP5* gene expression. Polymorphisms in *FKBP5* have been associated with post-traumatic stress disorder (PTSD)[4-10], depression[11], depressive episode recurrence[12], and bipolar disorder[13]. Notably, associations between *FKBP5* polymorphisms and psychiatric disorders have been somewhat variable among Caucasian and African-American populations[14,6].

Although these studies have outlined clear associations between FKBP5 and various affective disorders, mechanisms by which FKBP5 dysregulation may mediate symptom risk are still unknown. This relationship may be clarified through the study of intermediate phenotypes: discrete, heritable traits associated with genetic variance and disease phenotype[15]. Given the role of FKBP5 in endocrine response to stress, this gene is likely to influence maladaptive behaviors which comprise intermediate phenotypes that underlie stress-related psychopathology[16]. One candidate intermediate phenotype is biased attention to emotionally-valenced cues, which has been widely observed in anxiety[17] and depression[18] and linked to allelic variations in the serotonin transporter gene[19, 20]. There is some evidence to suggest the involvement of FKBP5 in this phenomenon. Multiple studies indicate that attention biases are closely associated with circulating levels of cortisol[21-25], and that these biases may be influenced by cortisol administration[24]. Given the role of the GR in modulating the body's response

to cortisol, attentional biases for emotional stimuli could be regulated through GR signaling, and thus moderated by polymorphisms in genes that influence the GR receptor complex, such as *FKBP5*.

Further, emerging evidence suggests that attention biases may be associated with alterations in brain function among individuals who hold increased genetic risk for psychopathology. For example, Thomason and colleagues found that children who carried specific allelic variants of the serotonin transporter gene (related to depressive vulnerability) demonstrated an attention bias toward fearful facial expressions and increased BOLD signal in parietal and paralimbic regions, relative to participants without this allele[26].

For *FKBP5* in particular, the hippocampus is an attractive target for brain-behavior phenotype investigations, given that this region has been associated with high GR density[27] and *FKBP5* expression; further, psychiatric disorders linked to *FKBP5* genetic status, particularly PTSD, have been associated with abnormal hippocampal volume[28-30] and function[31, 32]. The hippocampus has also been implicated in the processing of context during acquisition of conditioned fear responses and, in concert with prefrontal regions[33], participates in the appraisal of potential threat in new situations[34-36]. These hippocampal functions may be partly mediated by *FKBP5*, given the potential role of this gene in the stress response.

Thus, the goal of the present study was to investigate the relationship between an *FKBP5* polymorphism and a candidate cognitive phenotype, attention bias for threat cues, in highly-traumatized individuals, some of whom met criteria for Axis I psychopathology. We selected a putatively functional[37] *FKBP5* SNP that has been associated with anxious and depressive psychopathology: rs1360780. Recent experiments have shown that the rs1360780 “risk” allele is associated with increased transcription of *FKBP5* following stimulation by glucocorticoids, possibly by increasing binding of the risk-allele containing DNA sequence to TATA-box binding protein, a

transcriptional enhancer[37]. Increased *FKBP5* mRNA transcription in peripheral blood cells immediately after trauma exposure has been associated with a higher risk for subsequent PTSD[38].

Attention biases were characterized through behavioral and neural responses during functional MRI in a subset of these individuals. We tested the hypothesis that "risk" allele carriers would demonstrate significantly greater attention bias for threat and increased neural response in the hippocampus, relative to individuals without this allele. In addition, we conducted structural analyses within this region of interest to characterize the neural substrates of differential task response between individuals with and without the risk allele.

2. Methods

2.1 Participants

A total of 110 adult African-American men and women (gender and race self-identified) were enrolled as part of a larger study investigating genetic risk for stress-related disorders; study procedures were approved by the institutional review boards of Emory University School of Medicine and Georgia State University. Participants were recruited from the general medical clinics of a publicly-funded hospital, as detailed previously [4, 39]. Individuals were deemed eligible for participation if they could give informed consent and understand English, as determined by a study researcher. Participants were administered the Traumatic Events Inventory (TEI) to detail frequency and type of traumatic experiences, the PTSD Symptom Scale (PSS[40]), to measure presence and frequency of current PTSD symptoms, and the Structured Clinical Interview for DSM disorders (SCID[41]) to detect comorbid psychopathology such as Major Depressive Disorder or Bipolar Disorder. Demographic and clinical characteristics of this sample are detailed in eTable 1.

A subset of these participants (47 women) completed MRI; data from these (all traumatized) individuals were included in a prior study of PTSD[42], and were selected from the larger sample based on

inclusion/exclusion criteria for this study. Exclusion criteria for this study included current psychotropic medication use, medical or physical conditions that preclude MRI scanning; a diagnosis of bipolar disorder, schizophrenia or other psychotic disorder; medical conditions that contribute significantly to psychiatric symptoms; history of head injury or loss of consciousness for >5 minutes; history of neurological illness.

2.2 DNA Extraction, Genotyping, Quality Control

DNA was extracted from saliva collected in Oragene vials (DNA Genotek Inc., Ontario Canada) or from whole blood collected in EDTA tubes. DNA from saliva was extracted using the DNAdvance extraction kit (Beckman Coulter Genomics, Danvers MA) and DNA from blood was extracted using the E.Z.N.A. Blood DNA Midi Kit (Omega Bio-tek, Norcross, GA). All DNA for genotyping was quantified using PicoGreen (Invitrogen Corp., Carlsbad, CA) or NanoDrop (Thermo Fisher Scientific Inc., Waltham, MA) and normalized to a concentration of 5 ng/μl. DNA was plated into 384 plates at 10ng and dried down prior to performing the genotyping reactions.

For genotyping, 1ml of whole blood or 500ul of Oragene saliva were used for extraction. Genotyping of *FKBP5* SNP rs1360780 was conducted using a Taqman SNP Genotyping Assay along with Taqman Genotyping Master Mix (Applied Biosystems Inc., Foster City, CA). Alleles were discerned using the 7900HT Fast Real-Time PCR system. Negative controls and within- and across- plate duplicates were used for quality control. All negative controls passed QC. 40% of samples were genotyped in duplicate with no genotype discordants. The Taqman genotype call rate was 97%. Alleles were in Hardy-Weinberg Equilibrium (African-Americans only, p=0.19).

FKBP5 SNP rs1360780 was selected based on our prior studies with *FKBP5* polymorphisms within this population. We tested for associations under a risk=T-allele carrier model. Participants were categorized into two groups based on genotype; individuals with one or two copies of the T allele,

previously associated with psychopathology risk, were categorized in the "risk" group (TC/TT), whereas individuals without this allele (CC) were categorized in the "resilient" group.

2.3 Attention bias (dot probe) task description

A dot probe task was employed to measure attention biases for threat; this measure has reliably detected biases in prior studies of individuals with emotional disorders[17]. Task procedures have been previously described[43] (also detailed in Supplemental Materials), and trial structure is illustrated in Figure 1a; briefly, in each of 80 trials a central fixation cross appears for 500 ms, followed by a pair of face photographs (both of the same model) presented for 500 ms. Each pair comprises either one emotionally expressive (threatening/happy) and one neutral face or two neutral faces. After the offset of the faces, an asterisk replaces one of the faces for 1100 ms. Participants quickly indicate, with a forced-choice button press, whether the asterisk appeared on the left or right side of the screen. A modified version of this task, detailed elsewhere[42], was presented during neuroimaging.

Emotion bias scores were first calculated by subtracting response time to emotion-congruent stimuli (probes that replace happy or angry/threatening pictures) from response time to emotion-incongruent stimuli (probes that replace neutral pictures); these scores were decomposed into threat bias and happy bias scores, for all stimuli combined and separately for AA and C faces. Positive scores indicate attentional capture by the threat or happy cue and negative scores reflect attentional avoidance of this cue.

2.4 Statistical analyses of behavioral data

Univariate analyses of covariance were conducted to examine whether genotype status was related to attention bias for threatening faces in general (Threat bias), or for threatening Caucasian (Threat bias C) or African-American faces (Threat bias AA), after covarying for the presence of Bipolar Disorder, Major Depressive Disorder, total trauma exposure (TEI total score), and PTSD symptoms (PSS

total score). A similar analysis was conducted to examine potential associations with happy bias score. In addition, three separate factorial ANCOVAs were conducted to examine potential interactions of genotype, PTSD diagnosis, and trauma exposure (in childhood or adulthood) on threat bias score after covarying for depressive psychopathology. A final analysis was conducted to compare bias scores for CC, TC, and TT groups, after covarying for psychopathology. A threshold of $p<.05$ was used to determine statistical significance.

2.5 MRI acquisition and pre-processing, fMRI data analysis

Scanning took place in a Siemens 3-Tesla research-dedicated scanner. A high-resolution T1-weighted structural scan was acquired using a MPRAGE sequence (176 slices, FOV=256 mm cubic voxels; 1x1x1 mm slice; TR= 2600ms; TE= 3.02 ms; TI= 900ms; flip angle= 8°). During task administration, a total of 26 contiguous echo-planar, T2-weighted images parallel to the anterior-posterior commissure line were acquired (TR=2530 msec; TE=30 msec; FOV=240 mm; 64x64 matrix; 3.75x3.75x4.0 mm voxel). Statistical Parametric Mapping, version 5 (SPM5, Wellcome Department of Neurology, London, UK: http://www.fil.ion.ucl.ac.uk/spm/) was used for file conversion, image pre-processing and fMRI statistical analyses. Functional images were slice-time corrected (with a high-pass filter applied) and realigned to the first image to correct for motion. The mean of the realigned undistorted images was then co-registered with the structural T1 volume, spatially normalized to standardized Montreal Neurological Institute (MNI) space[44] based on the position of the anterior and posterior commissure and smoothed with an 8mm FWHM Gaussian kernel.

For each participant's data, a first-level, fixed-effects model was created with vectors for onset time of each condition, including threat/neutral, happy/ neutral, and neutral/neutral trials; each trial included face-pair presentation and probe. Box-car functions using 1, -1 contrast conventions were used to indicate voxels that had a higher activation level for the primary contrast condition, which was

activation to threat probe incongruent (i.e., trials in which the probe replaced the neutral face of a threat-neutral pair) minus threat probe congruent trials (trials in which the probe replaced the threat face of a threat-neutral pair), the same events that were used to calculate threat bias score. T-tests were then conducted to examine between-group differences in FKBP5 genotype and BOLD signal change to threat within ROIs. Although primary analyses were conducted with ROIs, brain-wide analyses were also conducted for completeness. First, a statistical threshold of $p<.005_{uncorrected}$ and an extent threshold of ≥5 voxels per cluster was applied to brain-wide analyses**.** Region of interest analyses were conducted with masks of the parahippocampal gyrus, created with the WFU Pickatlas Toolbox[45]; a p<.05 threshold, extent threshold of ≥ 5 voxels per cluster was used to determine statistical significance. A non-linear transformation (http://www.bioimagesuite.org/Mni2Tal/index.html) was used to convert coordinates from MNI to Talairach space[46] and a Talairach daemon[47] was used to localize anatomical coordinates of voxels associated with statistically significant patterns of BOLD activation.

2.6 Voxel based morphometry analysis

Using the BET toolkit in FSL[48], all structural images: were 1) skull-stripped (evaluated individually for errors); 2) segmented with FAST4[49]; 3) aligned to MNI152 standard space using affine registration; and 4) underwent nonlinear registration using FNIRT. Following alignment, a study-specific template was generated. The segmented images were then smoothed using an isotropic Gaussian kernel with a sigma of 3mm, and voxelwise GLM was carried out using permutation-based, non-parametric testing[50] to control for type I error. Statistical thresholding was set at $p<.001_{uncorrected}$, for ROIs.

2.7 Shape analyses

Global shape analysis (GSA) was performed using methods fully detailed in Supplemental Materials. Following skull extraction, images were fed through a GSA workflow that automatically extracted each brain into 56 distinct regions. Inhomogeneity correction of images was followed by

affine registration, non-linear registration to the LBP40 atlas[51], and subsequent automatic volume parcellation, during which each voxel in the brain was labeled[52]. The regional boundaries of the resulting labeled image were then converted into 56 distinct shapes[53], which underwent subsequent pre-processing, modeling, and analysis. A total of 5 shape metrics were used to perform group comparisons between the TC/TT and CC genotypes: volume, surface area, mean curvature, shape index, and curvedness. The resultant output of this analysis included sets of raw p-values and False-Discovery Rate (FDR) corrected p-values[54]. Supplementary Table 1 shows the standard intrinsic shape measures computed for each region.

Local shape analyses (LSA) of both hippocampi[53] were conducted in order to identify the specific vertices/subregions that demonstrated significant radial distance and/or displacement vector field variations from the study-defined mean hippocampal shape template[55]. In the LSA protocol, the structural attributes and cortical measures are calculated per-vertex on specific shape regions which are first co-registered across subjects to establish homologous anatomical features before statistically analyzing them against various subject demographic, clinical or phenotypic data[56]. To control for multiple comparisons testing, FDR-correction was applied. An atlas (Figure 3b) was used to identify anatomical locations of between-group differences[55].

3. Results

3.1 Group characteristics

Genotype data were not available for 5 participants due to insufficient quality of the DNA samples, leaving a total participant sample of 105. In this sample, participants reported experiencing a range of 0-10 traumatic experiences, with 7.7% of participants endorsing no trauma exposure. Approximately 6.7% of participants met criteria for a lifetime history of bipolar disorder, 13.3% met criteria for current MDD, and based on PSS items (in keeping with DSM-IV PTSD criteria), 41.2% met

criteria for PTSD. Within this sample, no significant differences (p>.05) emerged in demographic or clinical characteristics between CC and TC/TT genotype groups (eTable 1). Although significant differences were observed between genotype groups for psychotropic medication usage (p<.05, X^2=7.51), no significant differences in threat or happy bias score were observed between participants who were or were not taking psychotropic medication (p>.05). Within the imaging cohort, age and level of current PTSD symptoms were similar for risk and resilient genotypes. However, since incidence of trauma exposure differed significantly between groups (p<.05), this served as a covariate in subsequent analyses, in addition to PTSD symptoms. A comparison of clinical and demographic characteristics between the entire sample and the MRI cohort revealed no significant differences in age, PTSD symptoms, trauma exposure, household monthly income and educational level (p>.05).

3.2 Behavioral differences in attention bias to threat between FKBP5 (rs1360780) genotypes

Two other participants were excluded from behavioral data analyses due to a high number of skipped trials or inaccurate responses on trials (over 20%), leaving a total of 103 participants with valid behavioral data. Univariate tests including trauma exposure and presence of PTSD, MDD and Bipolar Disorder as covariates and three threat bias indices (1.threat bias score for both race types combined, and threat bias score for either 2. African-American, or 3. Caucasian faces) as dependent variables, were conducted. After covarying for trauma exposure and presence of PTSD, MDD and Bipolar Disorder, risk allele (TC/TT) carriers demonstrated a statistically significant ($F_{1,90}$=5.19, p<.05) bias toward threat (both AA and C races, combined; mean threat bias score=13.1, SD=37.2) compared to CC genotype (Mean threat bias score=-6.8, SD=46.2; see Figure 1b). These findings were verified with permutation testing, using PLINK software, version 1.07[57], to ensure that results did not depend on distributional assumptions; a total of 100,000 permutations were performed.

Separate univariate analyses revealed no significant genotype associations with happy bias (composite bias score, or separated by face race; p>.05). Further univariate analyses (including the same covariates) revealed no significant genotype effects for threat bias for Caucasian faces (p>.05), although a trend was observed for African-American faces ($F_{1,90}$=3.48, p=.065). Results from separate factorial ANCOVAs including psychopathology covariates and threat bias score as the dependent variable indicated no significant interactions of genotype status and 1) childhood sexual or physical abuse or 2) adult trauma exposure (presence or absence, as reported in the TEI). A univariate analysis including genotype and PTSD status as factors did not reveal significant interactions of these variables on threat bias score, p>.05. Genotype contributed significantly to this model (p<.05), whereas PTSD status did not, although a trend was observed (p=.17) . A final factorial ANCOVA revealed that, when CC, TC, and TT genotype groups were examined separately, no significant main effects were observed after covarying for psychopathology (possibly due to limited sample size) although a trend was observed ($F_{1,90}$=2.47p=.09); individuals in the CC genotype group demonstrated a slight bias away from threat (Mean bias score=-6.78, SD=46.21) whereas both TC (Mean bias score=13.25, SD =39.26) and TT (Mean bias score=12.71, SD=41.40) groups demonstrated a bias toward threat.

3.3 fMRI reveals differences in hippocampal activation to threat between FKBP5 genotypes

Due to excessive motion (7), and/or brain parenchyma abnormalities (4), 11 participants were excluded from MRI data analyses, leaving a total of 36 participants (10 CC, 26 TC/TT); another (TC/TT) participant was excluded from fMRI (but not structural MRI) analyses, due to a high number of skipped trials (over 20%) on the task. Behavioral data from the cohort of participants who completed the fMRI version of the task (n=35) yielded no significant genotype effects on threat bias, regardless of face race (p>.05).

In a whole-brain analysis for the threat incongruent versus threat congruent trials contrast condition, subjects with the TC/TT genotype demonstrated significantly greater BOLD signal in the right

hippocampus and left parahippocampal gyrus, as compared to the CC genotype ($p<.005_{uncorrected}$; see eTable 2, Figure 2); these findings did not change significantly when hippocampal volume was entered as a covariate; see Supplementary Table 2. The CC genotype, relative to TC/TT genotype, did not demonstrate greater BOLD signal in hippocampal regions (see eTable 2).

Subsequent ROI analyses confirmed these findings, with the TC/TT genotype group demonstrating increased activation in the right and left hippocampus relative to the CC group ($p<.05$; see eTable 2). BOLD signal values were extracted from 6mm spheres within these regions and entered into two separate, 3-level hierarchical regressions, with trauma incidence (TEI total; level 1), PTSD symptoms (PSS total; level 2) and genotype (level 3) serving as predictors of BOLD signal in these regions. Trauma exposure did not predict a significant amount of variance in BOLD signal within the right (Beta = -.15, R^2=.02, p>.05) or left hippocampus (Beta=-11, R^2=.01, p>.05); when added to this model, PTSD symptoms likewise did not predict a significant proportion of variance in BOLD signal within the right (Beta = .24, R^2 =.08, p>.05) or left hippocampus (Beta=.03, R^2=.02, p>.05). However, when added in the third step of these models, *FKBP5* genotype accounted for a significant amount of variance in BOLD signal for the right (Beta=-.44, R^2 = .28, p<.05) and left hippocampus (Beta=-.46, R^2 = .21, p<.05). There was no direct association between threat bias score and neural response within ROIs.

3.4 Voxel-based morphometry reveals no differences in hippocampal density between FKBP5 genotypes

At the $p< .001_{uncorrected}$ statistical threshold, there were no between-group differences in gray matter density of the hippocampus.

3.5 Morphological analysis reveals differences in hippocampal shape between FKBP5 genotypes

The global shape analysis revealed some between-group differences in shape index of the left hippocampus ($p<0.05_{uncorrected}$). Additionally, local shape analysis (LSA) results (Figure 3b) revealed significant between-group differences in displacement vector field measure in the left and right

hippocampus ($p<0.001_{FDR\text{-}corrected}$). Relative to the right hippocampus, a larger area of the left hippocampus displayed between-group spatial displacement vector metric differences, predominantly in the CA1 region. The genotypic effect on hippocampal surface area was reduced ($p>.05$) when TEI score and/or hippocampal volume were included as covariates.

To ensure that these genotypic effects were not present in similar cortical and subcortical regions, these LSAs were conducted on the left and right insula and caudate. None of these regions showed statistically significant genotypic effects ($p> .05$).

4. Discussion

The present study investigated associations among genotypic variants of a putatively functional *FKBP5* SNP, rs1360780, and response to threat cues in an attention bias paradigm. In addition to investigating behavioral response, we examined corresponding differences in function and structure within a brain region that appears to be affected by *FKBP5* gene expression, the hippocampus. Our findings from these parallel behavioral, functional and structural neuroimaging datasets revealed that, relative to individuals without this allele, carriers of the 'T' allele (which has been previously linked to incidence of psychopathology) demonstrated: 1) an attentional bias toward threat; 2) increased hippocampal activation to threat; and 3) differences in hippocampal shape.

Preferential attentional processing of threat cues has purported relevance to the development and maintenance of pathological anxiety[58]. The findings from the present study document, for the first time, an association between *FKBP5* polymorphism and threat bias. An increasing number of studies indicate that *FKBP5* genetic variability is linked to behaviors that reflect atypical or dysregulated emotion processing, including aggression[59] and suicidality[60]. Furthermore, earlier findings reflected interactive effects of genetics and environmental stress (i.e., childhood abuse), that is, the genetic effects were most pronounced in the presence of high levels of prior stress or trauma[4]. Notably, we

found that the relationship between *FKBP5* allelic variance and attention bias for threat remained significant after controlling for variance associated with psychopathology, as well as trauma exposure, and no interactive effects were observed among *FKBP5* genotype, trauma exposure and post-traumatic psychopathology. Thus, the associations observed between *FKBP5* genotypic variance and attention bias were not better accounted for by variance linked to environmental stress or psychopathology. It is also possible that, when considering the relationship between *FKBP5* genotype and attention bias for threat, the effects of maltreatment, exposure to traumatic events in adulthood and a relatively high amount of life stressors (e.g., occupational stress, racial discrimination, bereavement, divorce) may be additive, and not interactive, in nature. Although the cross-sectional design of this study precludes definitive statements regarding risk, our findings suggest that *FKBP5* risk allele carriers, compared to individuals without this allele, are more likely to demonstrate a heightened vigilance for ostensibly mild threat cues. An attentional preference for these mild threat cues, particularly among individuals with trait anxious characteristics, indicates increased susceptibility to psychopathology under stressful conditions.

In this study, risk allele carriers (who are likely to demonstrate increased transcription of *FKBP5* following stress exposure), also demonstrated heightened hippocampal activation in response to threat, specifically, to a condition that approximates threat bias. To our knowledge, no previous studies have examined associations between *FKBP5* polymorphisms and hippocampal function, particularly, response to threat. However, prior data identify *FKBP5* as a putative modulator of physiological stress response systems, suggesting that these associations merit attention. Our findings indicate that increased hippocampal responsiveness to threat cues was selectively associated with risk allele carrier status, implicating this gene in neural mechanisms of threat evaluation. Given the density of GRs in the hippocampus, and the role of *FKBP5* in regulating GR function, the increased hippocampal activation may represent, in part, *FKBP5*-mediated sensitization of neural systems to external threat signals. Risk allele carriers may have a lower threshold for perceiving threat in the environment compared to

individuals without this allele, and thus may demonstrate heightened responsiveness in neural circuits mediating threat evaluation. Notably, earlier studies of pathologically-anxious individuals have revealed enhanced activation in hippocampal regions[61], as well as frontal regions with limbic connections, during expression of attention biases[33, 62]. Thus, the heightened hippocampal response to mild threat cues observed in risk allele carriers could represent higher baseline responsiveness in neural networks engaged during threat evaluation in these individuals.

These functional differences may also be understood in the context of structural differences. Although no differences in hippocampal density were noted, risk allele carriers demonstrated differences in hippocampal shape compared to individuals without this allele, specifically, in the CA1 region. Abnormalities in hippocampal structure, particularly, the CA1 region[63-64], have been frequently noted in individuals with stress-related disorders[65-66]. This region may be particularly susceptible to the effects of stress[67], and changes in CA1 plasticity could be partially mediated by *FKBP5* mechanisms, given previous observations of *FKBP5* mRNA up-regulation following stress exposure[68]. Individuals within our study population have experienced relatively high incidence of trauma exposure and psychosocial stress, which has been linked to suppressed neuronal proliferation in the hippocampus[69]. These conditions are likely to interact with genetic vulnerability, and structural differences in the hippocampus may be particularly apparent among individuals who carry a "risk" genotype, one that has been previously associated with adverse behaviors and diagnostic outcomes.

In addition, there were no significant genotypic effects in other regions examined, providing evidence to support specificity of *FKBP5* genetic effects on the hippocampus. Thus, the structural differences observed here indicate a putative morphological substrate for the differential response to threat observed between genotype groups. However, given that genotypic effects were no longer apparent after including trauma exposure to the statistical model, replication in a larger sample is needed to ascertain the robustness of these findings.

Other study limitations must be noted. First, given that only female face stimuli were used in this version of the dot probe, it was impossible to investigate potential interactive effects of gender and attentional biases. Similarly, a lack of Caucasian participants in this study prohibited examination of stimulus- by participant-race interactions and their effects on attentional biases. Also, our imaging cohort included only female participants, thus precluding investigation of gender-specific differences in hippocampal response or structure. In addition, some research suggests that FKBP5 may be a target of progesterone[70]; unfortunately, endocrine and menstrual cycle data were unavailable in the presence study to examine potential relationships with behavioral and neural response. These factors merit consideration in future investigations. Although hypothesis testing was limited to a particular ROI, the relatively small size of our imaging cohort (and particularly, the size of one genotype group) increases the likelihood of type II error. Further, meditational analyses would provide an ideal statistical approach for determining indirect effects of hippocampal function and size on attentional bias for threat; however, our limited sample size did not confer sufficient statistical power to detect these effects. Thus, these neuroimaging findings should be viewed as preliminary and warrant confirmation in genetic-neuroimaging studies conducted with larger samples.

In sum, we observed that genotype status for a putatively functional *FKBP5* SNP was associated with differential behavioral and hippocampal response during performance of an attention bias task. Structural differences in hippocampal formation served to further distinguish these genotype groups. Taken together, these data may represent behavioral and neural markers of *FKBP5* function, and merit further investigation in future studies of genetic risk for affective psychopathology.

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Figure Legends

Figure 1. Attention bias (dot probe) task. a) ***Trial structure.*** Rows illustrate trials that were used to calculate threat bias and as fMRI contrast conditions. The top row displays trials in which the probe appears on the opposite side of the threatening expression (threat probe-incongruent); the bottom row displays trials in which the probe appears on the same side of the threatening expression (threat probe-congruent). b) ***Attention bias to threat as a function of FKBP5 genotype: TC/TT genotypes demonstrate attentional bias toward threat, compared to CC genotype***. Chart illustrates mean attention bias score (error bars indicate standard error of the mean), for threat faces (both races, combined) and separated by African-American (AA) and Caucasian (C) race type, as a function of genotype group. Asterisk indicates p<.05.

Figure 2. **FKBP5 polymorphism is associated with differential hippocampal activation during attention to threat.** a) Statistical parametric maps of left and right hippocampus activation during the processing of threat probe-incongruent versus threat probe-congruent faces in TC/TT > CC genotype. Activations are shown overlaid onto a canonical T1 magnetic resonance image. The colored bar represents *t* scores for activations. Maximally activated voxels from the left parahippocampal gyrus (x, y, z: -36,-35,-8) and right hippocampus (x, y, z: 36, -24, -12), $p < 0.05_{SVC\_FWE}$. Data are reported using the coordinate system of Talairach and Tournoux. b) Genotype differences in averaged BOLD signal (contrast time series extracted from 6mm spherical ROIs) to this contrast condition.

Figure 3. FKBP5 polymorphism is associated with differences in hippocampal shape. a) Local shape analyses of left and right hippocampus. Smaller p-values (FDR-corrected) reflect greater spatial displacement for TC/TT versus CC genotypes. b) Cross-sectional views of the 3D hippocampal atlas (Yushkevich et al., 2009) used for reference. Abbreviations: CA = cornu ammonis; DG = dentate gyrus; H = hilum; SM= stratum moleculare; VHS = vestigial hippocampal sulcus.

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Table 1. Demographic and Clinical Characteristics

(N=105, all African-American)

	CC (n= 36)	TC/TT (n= 69)	
	Mean (SD)		*p*
Age	43 (12.2)	40.2 (12.5)	.36
PSS total	17.2 (13.3)	14.7 (12.3)	.43
TEI total	3.9 (2.7)	3.6 (2.5)	.57
Females (n)	31	55	.74
Education	**%**	**%**	
< 12th grade	15.8	25.4	.71
12th grade/high school graduate/GED	42.1	45.1	
Some college/technical school	28.9	19.7	
College/technical school graduate	13.1	9.8	
Monthly Income			.48
$0 – 249	26.3	37.7	
$250 – 499	5.3	5.8	

$500 – 999	42.1	27.5	
$1000-1999	18.4	24.6	
$2000+	7.9	4.3	
Major Depressive Disorder (current)	16.7	11.6	.47
Bipolar Disorder (lifetime history)	5.6	7.2	.10
Primary Psychotic Symptoms (past month)	21.9	18.8	.13
Taking Psychotropic Medications	32.4	10.6	.006*
MRI Cohort (N=36, all female)	CC (n=10)	TC/TT (n=26)	
	Mean (SD)		
Age	40 (13.5)	35.2 (13.4)	.34
PSS total	16.5 (14.2)	13.6 (10.9)	.51
TEI total	2.6 (1.7)	4.7 (2.7)	.03*
Education	**% within group**	**% within group**	.87
< 12th grade	44.4	12.5	
12th grade/high school graduate/GED	11.1	45.9	
Some college/technical school	22.2	25	
College/technical school graduate	22.2	16.6	
Monthly Income			.08

$0 – 249	0	17.4
$250 – 499	22.2	13
$500 – 999	22.2	34.8
$1000-1999	22.2	34.8
$2000+	33.3	0

*p<.05

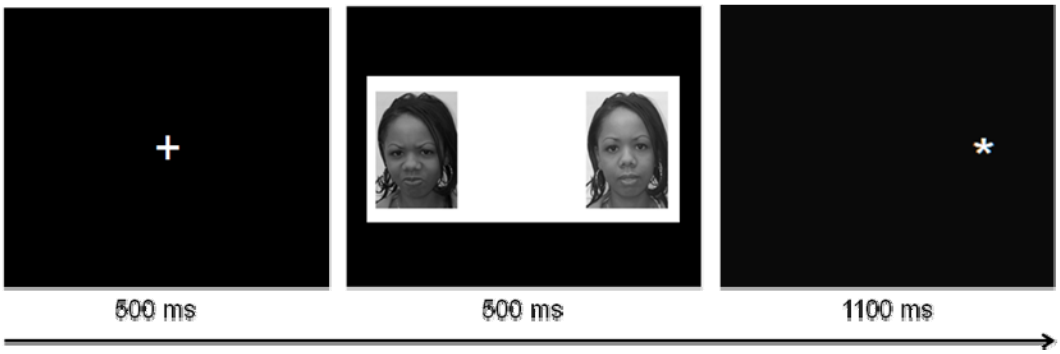
Table 2.

Anatomical locations of activation to threat probe-incongruent versus threat probe-congruent trials between CC and TC/TT genotype groups (p<.005)

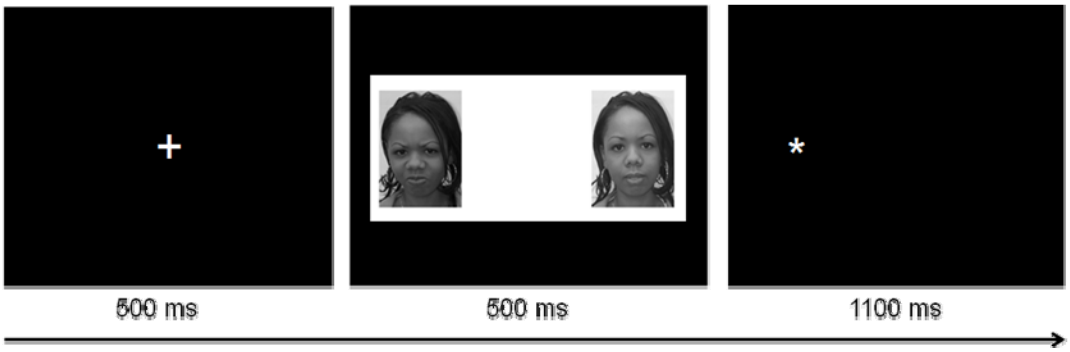
x	y	z	k	t	Brodmann Area	Anatomical location
Brain-wide analysis						
TC/TT > CC						
36	-24	-12	5	3.81		Hippocampus
28	-55	-17	5	3.28		Cerebellum
-36	-35	-8	5	2.91	36	Parahippocampal gyrus
-32	-28	-12		2.89		Hippocampus
CC > TC/TT						
4	6	33	46	4.42	24	Cingulate gyrus
8	-13	41		2.92	24	
12	-56	54	13	4.06	7	Precuneus
-12	-76	37	9	3.60	7	
55	-23	16	5	3.22	40	Postcentral gyrus
48	-22	34	15	3.19	2	
55	-25	34		3.14	2	
4	-52	43	5	3.17	7	Precuneus
20	-72	37	10	3.08	7	
8	-76	37		2.92	19	Cuneus
ROI analysis						
TC/TT > CC						
32	-24	-12	12	3.45		Hippocampus
-32	-28	-12	17	2.89		Hippocampus
-32	-5	-17	5	2.83		Parahippocampal gyrus
36	-43	-5	5	2.45	19	Parahippocampal gyrus

x, y, z Talairach coordinates

a)



Threat probe-incongruent trial



Threat probe-congruent trial

b)

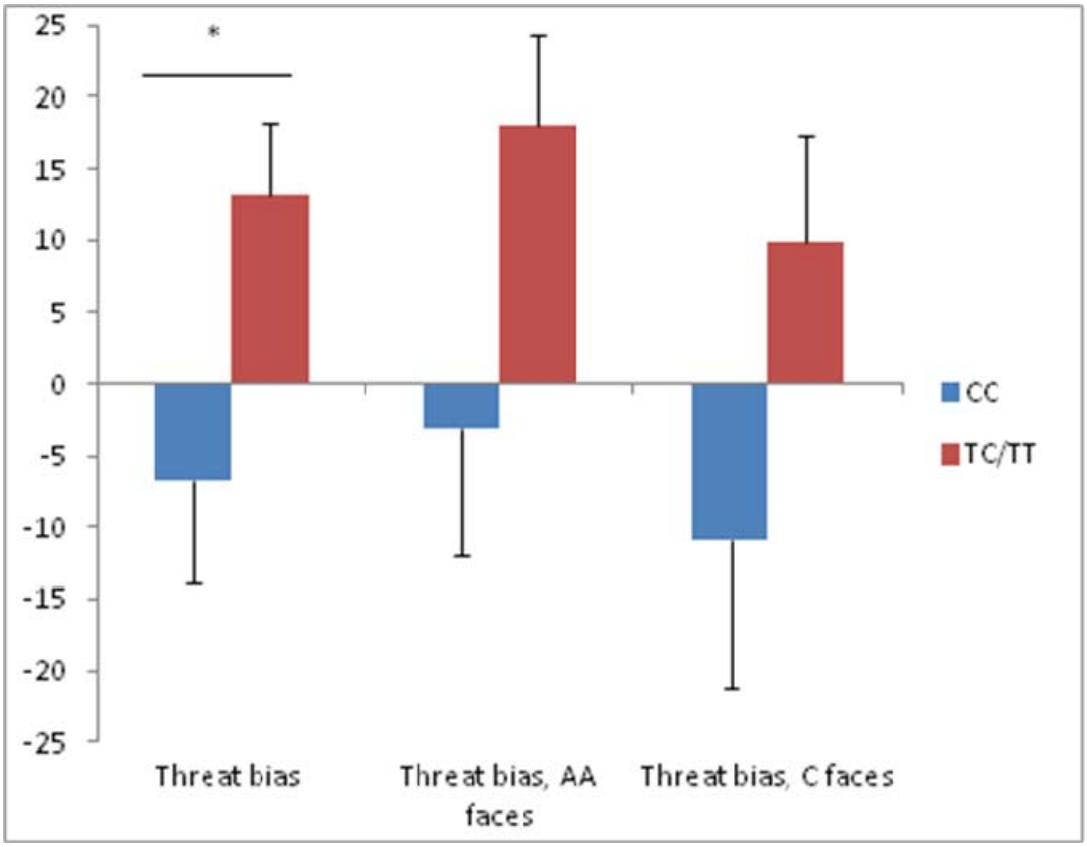
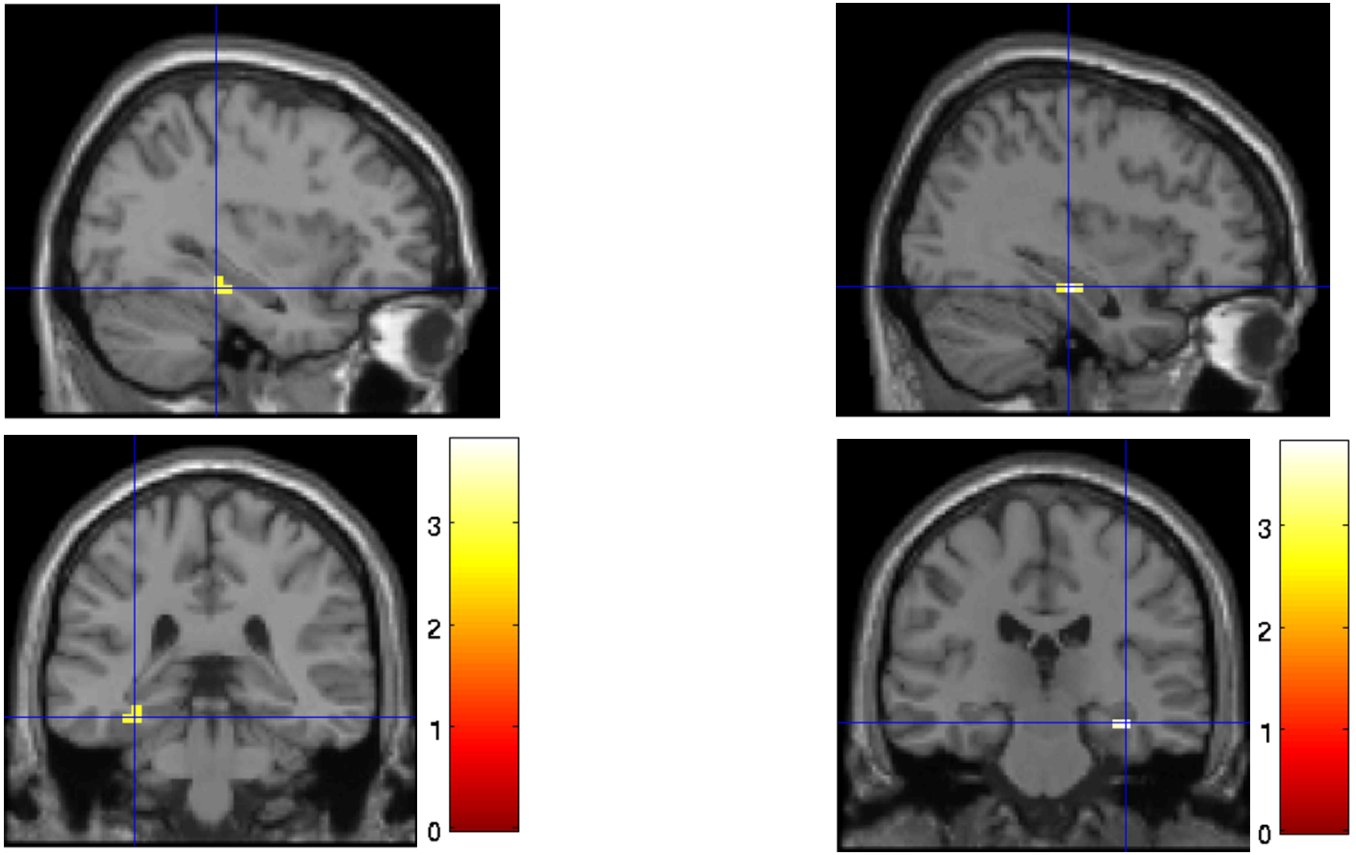


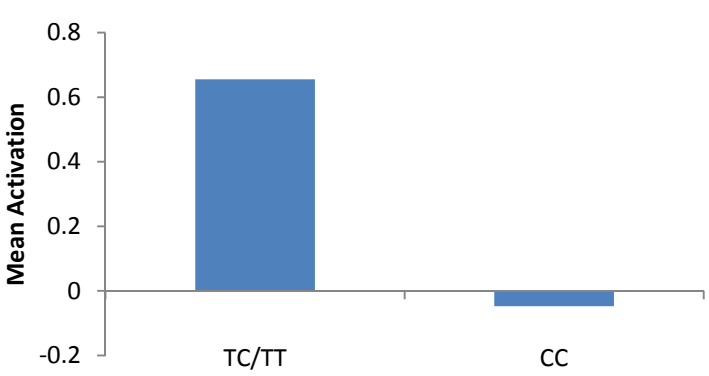
Figure 1. **a) Attention bias (dot probe) task trial structure.** Rows illustrate trials that were used to calculate threat bias and as fMRI contrast conditions. The top row displays trials in which the probe appears on the opposite side of the threatening expression (threat probe-incongruent); the bottom row displays trials in which the probe appears on the same side of the threatening expression (threat probe-congruent). b) **Attention bias to threat as a function of FKBP5 genotype: TC/TT genotypes demonstrate attentional bias toward threat, compared to CC genotype**. Chart illustrates mean attention bias score (error bars indicate standard error of the mean), for threat faces (both races, combined) and separated by African-American (AA) and Caucasian (C) race type, as a function of genotype group. Asterisk indicates $p<.05$.

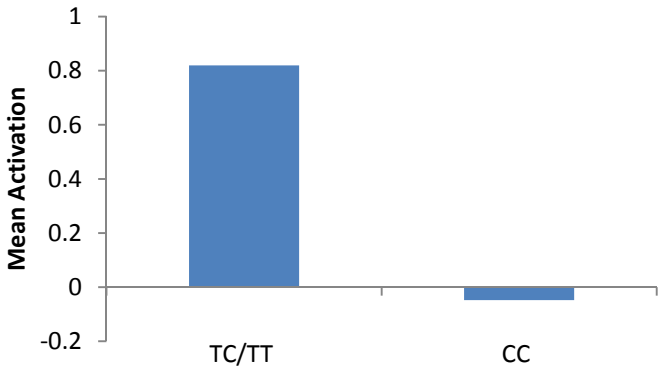
Figure 2.

a.


3
2
1
0
3
2
1
0

b.


Mean Activation
0.8
0.6
0.4
0.2
0
-0.2
TC/TT
CC


Mean Activation
1
0.8
0.6
0.4
0.2
0
-0.2
TC/TT
CC

a) Statistical parametric maps of left and right hippocampus activation during the processing of threat probe-incongruent versus threat probe-congruent faces in TC/TT > CC genotype. Activations are shown overlaid onto a canonical T1 magnetic resonance image. The colored bar represents *t* scores for activations. Maximally activated voxels from the left parahippocampal gyrus (x, y, z: -36,-35,-8) and right hippocampus (x, y, z: 36, -24, -12), $p < 0.05$ (SVC). Data are reported using the coordinate system of Talairach and Tournoux .

b) Genotype differences in averaged BOLD signal (contrast timeseries extracted from 6mm spherical ROIs) to this contrast condition.

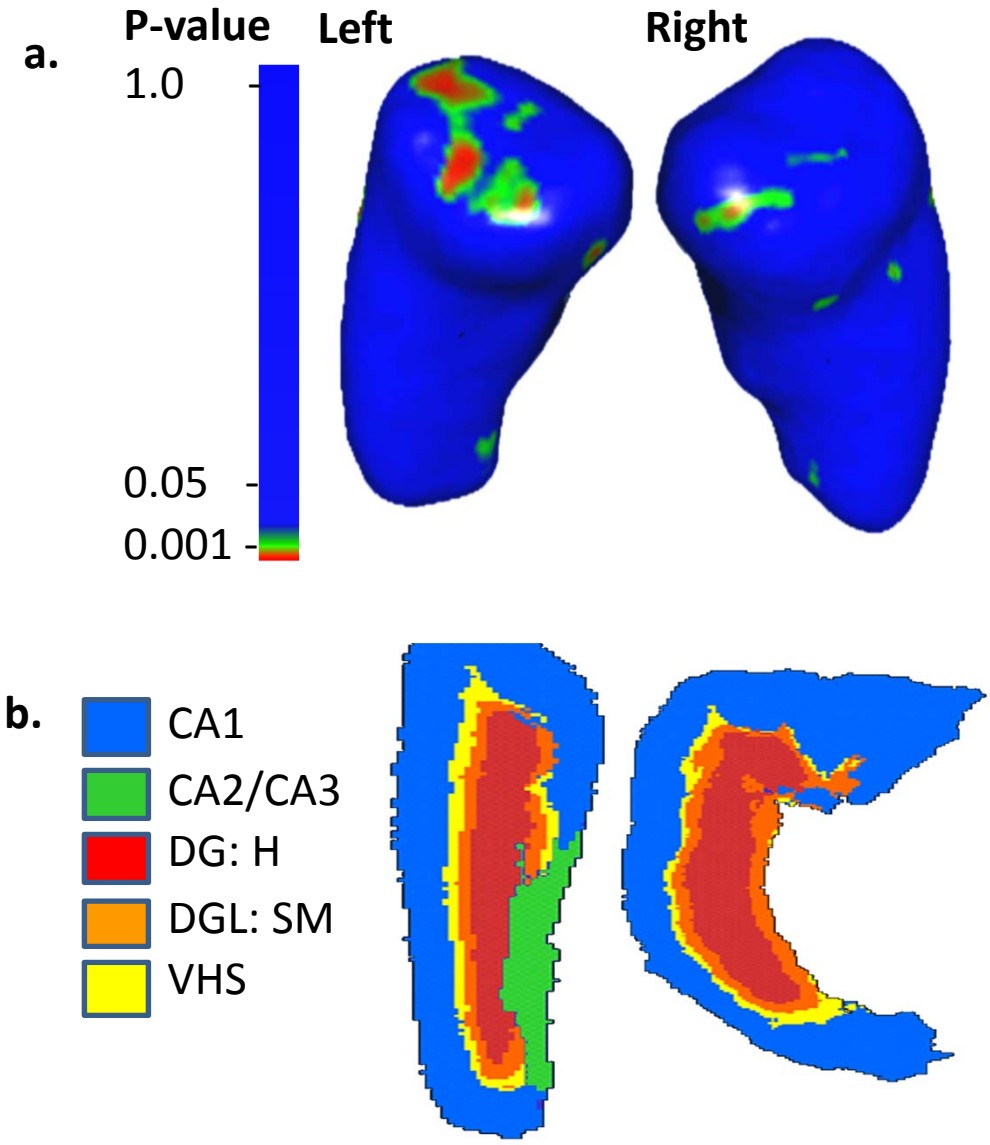

a.
P-value
1.0
0.05
0.001
Left
Right
b.
CA1
CA2/CA3
DG: H
DGL: SM
VHS

Figure 3. **FKBP5 polymorphism is associated with differences in hippocampal shape.** a) Local shape analyses of left and right hippocampus. Smaller p-values (FDR-corrected) reflect greater spatial displacement for TC/TT versus CC genotypes. b) Cross-sectional views of the 3D hippocampal atlas (Yushkevich et al., 2009) used for reference. Abbreviations: CA = cornu ammonis; DG = dentate gyrus; H = hilum; SM= stratum moleculare; VHS = vestigial hippocampal sulcus.