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Neural and genetic markers of vulnerability to post-traumatic stress symptoms among survivors of the World Trade Center attacks

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Although recent research has begun to describe the neural and genetic processes underlying variability in responses to trauma, less is known about how these processes interact. We addressed this issue by using functional magnetic resonance imaging to examine the relationship between posttraumatic stress symptomatology (PTSS), a common genetic polymorphism of the serotonin transporter [5-HTT (5-hydroxy tryptamine)] gene and neural activity in response to viewing images associated with the 9/11 terrorist attack among a rare sample of high-exposure 9/11 survivors ($n = 17$). Participants varied in whether they carried a copy of the short allele in the promoter region of the 5-HTT gene. During scanning, participants viewed images of the 9/11 attack, non-9/11 negative and neutral images. Three key findings are reported. First, carriers of the short allele displayed higher levels of PTSS. Second, both PTSS and the presence of the short allele correlated negatively with activity in a network of cortical midline regions (e.g. the retrosplenal and more posterior cingulate cortices (PCCs)) implicated in episodic memories and self-reflection when viewing 9/11 *vs* non-9/11 negative control images. Finally, exploratory analyses indicated that PCC activity mediated the relationship between genotype and PTSS. These results highlight the role of PCC in distress following trauma.

Keyword: post-traumatic stress; posterior cingulate cortex; polymorphism; 9/11; trauma; fMRI

INTRODUCTION

Much research has examined the neural (Etkin and Wager, 2007; Shin and Liberzon, 2010) and genetic (Xie *et al.*, 2009; Caspi *et al.*, 2010; Shin and Liberzon, 2010) processes that underlie variability in the way individuals respond to trauma. Comparatively less work has examined how these processes interact. We addressed this issue by examining the interrelationship between post-traumatic stress symptomatology (PTSS), a common genetic polymorphism of the serotonin transporter [5-HTT (5-hydroxytryptamine)] gene and neural activity in response to viewing images associated with the 9/11 terrorist attack among a small but rare sample of high-exposure 9/11 survivors.

Research on post-traumatic stress disorder (PTSD) suggests that functional abnormalities in brain regions that support the generation and regulation of emotion (Phan *et al.*, 2006) and self-referential processing (Raichle *et al.*, 2001; Buckner *et al.*, 2008) underlie individual differences in the way people respond to trauma (Britton *et al.*, 2005; Etkin and Wager, 2007; Jovanovic and Ressler, 2010; Shin and Liberzon, 2010; Lanius *et al.*, 2011). For example, previous studies (Liberzon *et al.*, 1999; Pissiota *et al.*, 2002; Lanius *et al.*, 2011; Whalley *et al.*, 2013, but see Sartory *et al.*, 2013) have shown that when presented with trauma-related reminders, individuals with PTSD display deactivations in a network of cortical midline regions known to be involved in emotion regulation, self-reflection and episodic memory (Raichle *et al.*, 2001; Schacter *et al.*, 2007; Buckner *et al.*, 2008), including the medial pre-frontal and posterior cingulate cortices (PCCs) and the precuneus. Deactivations in this network distinguish PTSD symptomatology from other anxiety disorders, such as specific phobia and social anxiety, which are often associated with hyperactivation of regions involved in generating fear states (e.g. amygdala and insula; Nielsen *et al.*, 2005; Etkin and Wager, 2007). The deactivation of these midline regions among people with PTSD has been linked to maladaptive emotion regulation, such as autonomic blunting or attentional avoidance (Foa and Kozak, 1986; Etkin and Wager, 2007; Shin and Liberzon, 2010), and the phasic experience of emotional numbness (Raichle *et al.*, 2001; Nielsen *et al.*, 2005; Buckner *et al.*, 2008).

Similar to other anxiety disorders, PTSD also has been related to abnormalities in the brain's serotonergic system (Bremner *et al.*, 1999; Pissiota *et al.*, 2002; Lanius *et al.*, 2003; Koenen *et al.*, 2005) and responds to treatment with selective serotonin reuptake inhibitors (Lesch *et al.*, 1996). In addition, individuals carrying a copy of the short allele in the promoter region of the 5-HTT gene, which is associated with reduced serotonin transporter availability and function, display elevated rates of PTSD (Lee *et al.*, 2005; Canli and Lesch, 2007; Green *et al.*, 2008; Grabe *et al.*, 2009; Koenen *et al.*, 2009; Xie *et al.*, 2009; Hyde *et al.*, 2011; Morey *et al.*, 2011).

Taken together, prior studies suggest that individuals carrying the short allele in the 5-HTT gene are at a greater risk of developing PTSS following exposure to trauma[1]. It is unknown, however, how this genetic risk factor is related to patterns of neural activity in midline

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[1] Although it is unlikely that any specific gene is uniquely associated with PTSD, there is growing evidence that the influence of genes on behavior can be realized through gene—environment interactions (Caspi *et al.*, 2010).

regions previously observed in individuals suffering from PTSD. To our knowledge, no study has examined the links between PTSS, neural activation in the midline regions and 5-HTT variability.

To address this issue, we asked a sample of high-exposure 9/11 survivors who displayed various levels of PTSS during the years since the 9/11 attack to view a series of photographs related to the attack, as well as negative control images of non-9/11 events, while we monitored their neural activity using functional magnetic resonance imaging (fMRI). Each participant provided us with a saliva sample, which allowed us to assess whether they possessed a short allele in the promoter region of the 5-HTT gene. Thus, three types of information were obtained for each participant: their PTSS levels at 7 months, 18 months and 6 years following the 9/11 attack, their 5-HTT makeup and their relative neural activity in response to images of the 9/11 attacks. Although only a relatively small number of 9/11 survivors was available for this study, these data offered us a unique opportunity to examine the relationship between two potential biomarkers of dysfunctional responses to trauma (neural activity and 5-HTT makeup) and their respective links to PTSS in response to a traumatic event of national importance.

We tested three hypotheses. First, we predicted that PTSS would be positively related to the presence of a short allele in the serotonergic 5-HTT genotype. Second, we hypothesized that PTSS would be inversely related to activity in cortical midline regions in response to images of self-experienced (9/11) trauma *vs* control images of negative non-9/11 events. Finally, we explored whether midline activity mediated the predicted relationship between genetic variability and PTSS to begin to explore whether a heritable brain-based biomarker might serve as a possible PTSD endophenotype.

METHODS

Participants

Participants were recruited from a larger group of 52 survivors of 9/11 who were either in the World Trade Center (WTC) or at the most within four blocks of the towers at the time of attack. The recruitment procedure and selection criteria for the larger group are reported elsewhere (Bonanno *et al.*, 2005, 2006). Valid contact information was confirmed for 37 individuals. Prospective participants were informed about the exclusion criteria, including inability to speak English or any of the following conditions: dependence on substances (other than nicotine) and neurological and medical conditions that might alter cerebral function. Twelve of the individuals contacted immediately declined participation. Out of the 25 expressing interest, 6 dropped out before arriving at the site and 1 person was excluded at the site because of language problems. Finally, one participant was excluded due to technical issues during scanning. The final sample[2] (9 males; $M_{age} = 45.6$, s.d.$_{age} = 12$) consisted of 16 European American and 1 Asian participants (Table 1 displays 5-HTT genotype distribution and demographics).

Before participating, all participants gave informed consent in accordance with the Columbia University Institutional Review Board. They were paid $250 for their participation.

Post-traumatic stress symptoms

PTSS was assessed using the Post-traumatic Stress Disorder Symptoms Scale (PSS-SR; (Foa *et al.*, 1993) at three time points: 7 months following the attack (wave 1), 18 months after the attack (wave 2) and 6 years after the attack (wave 3: at the time of this experiment). This scale consists of 17 items that ask people to self-report PTSD symptoms that correspond to those listed in the Diagnostic and Statistical Manual of Mental Diseases III-R (DSM-III-R) (APA, 1987). The PSS-SR has adequate internal consistency ($\alpha = .91$) and concurrent validity (Foa *et al.*, 1993). In this study, participants were asked to assess the frequency with which they experienced each item on the PSS-SR in the past month using a 0 (not at all or only one time) to 3 (5 or more times per week/almost always) scale. PTSS scores were significantly correlated across all three waves ($\alpha = .86$). Therefore, we averaged across time and used a composite PTSS score in our analyses to enhance the reliability of this instrument. Mean PSS-SR for the sample ($M = 17.00$, s.d. $= 18.26$) was above the clinical cut-point for PTSD (14, Coffey *et al.*, 2006).

Genotyping

Samples for DNA extraction were obtained from saliva using the protocol and reagents in the Oragene sample collection kit (DNA Genotek Inc., Kanata, Canada). Following extraction, DNA yields were determined spectrophotometrically by absorbance at 260 nm. For the genotyping of the serotonin-transporter-linked polymorphic region (5HTTLPR), we followed an amplification protocol based on Lesch *et al.* (1996) using oligonucleotide primers corresponding to the nucleotide positions −1416 to −1397 of the 5-HTT upstream region (5′-GGCGTTGCCGCTCTGAATGC-3′) and −910 to −888 (5′-GAGGGACTGAGCTGGACAACCAC-3′) that amplify a 484-bp 'short' allele and/or a 528-bp 'long' allele. Polymerase chain reaction (PCR) buffer and deoxynucleotide triphosphates (dNTPs) were obtained from QIAGEN and used at recommended concentrations for a 20 µl PCR reaction containing 50 ng of genomic DNA, 100 ng of each primer, 10-mmol/l Tris-hydrochloride (pH 8.3), 50-mmol/l potassium chloride, 1.5-mmol/l magnesium chloride, 0.01% gelatin, 2.5 mmol/l of each dNTP (including deoxyguanosine triphosphate (dGTP)/7-deaza-2′-dGTP:dGTP) and 0.8 U of AmpliTaq DNA polymerase (Promega, Madison, WI). Reactions were processed in a PTC-100 Programmable Thermal Controller (MJ Research) outfitted with a heated lid for oil-free amplifications. A touchdown PCR cycling regimen was used to automatically optimize the hybridization stringency. Gel electrophoresis in 1.5% Metaphor agarose followed by staining in ethidium bromide was used to resolve and visualize DNA fragments. Following conventional grouping of the genotypes, participants carrying a copy of the short allele (short/short or short/long genotype) in the promoter region of the serotonin transporter 5-HTT gene were combined to form the s-carrier group ($n = 11$) and were compared with l/l homozygotes (Caspi *et al.*, 2003; Lee *et al.*, 2005).

Stimuli

Each participant viewed 90 photos; 30 depicted scenes from the events of 9/11 (e.g. burning WTC towers; people jumping from the towers), 30 depicted negatively valenced non-9/11 images that were selected (to the extent possible) to match the 9/11 scenes (e.g. pictures depicting the aftermath of environmental disasters; burning buildings and fleeing people) and 30 were neutral images (e.g. intact buildings and people engaged in affectively neutral behaviors with neutral expressions). The

Table 1 5-HTT genotype distribution and demographics

	l/l ($n = 6$)	l/s, s/s ($n = 11$)
Age, mean (s.d.)	47 (12)	44 (12)
Female/Male	1/5	7/4
Ethnicity	6 Caucasian	10 Caucasian, 1 Asian

[2] The sample included 4four left-handed participants, evenly divided between carriers of the short and two long alleles. The results were not altered by including handedness as a covariate or excluding two participants who were medicated with anxiolytic (Xanax) and anti-depressive (Wellbutrin) drugs. Neither did therapy treatment ([reported at wave 2 (18th months post 9/11)] affect the results.

9/11 images were taken from the internet and the non-9/11 negative and neutral images were drawn from the International Affective Picture System (Lang *et al.*, 2008). Neutral images were included primarily to provide participants with a break from viewing highly arousing negative images and to prevent habituation.

Task

Participants were asked to attend to each presented image and allow for their thoughts and feelings to flow naturally. Each trial began with a fixation cross that appeared for 3000 ms. Next, one of the three types of images was presented randomly for 5000 ms. During the last 2000 ms of viewing each image, participants were asked to respond to the question, 'How do you feel right now?' ranging from very bad (1) to neutral (3) to very good (5). The time between the fixation cross and inter-trial interval (ITI) was systematically varied between 1000 and 15 000 ms (mean 7000 ms) to enhance recovery of the blood oxygen-level-dependent signal in response to image presentation. The functional run was divided into two sessions of 45 images each, interrupted by a brief pause.

Data acquisition and analysis

Stimulus presentation was controlled using E-Prime software (PST Inc.). A liquid crystal display (LCD) projector displayed stimuli on a back-projection screen mounted in the scanner suite. Responses were made with the right hand on a five-finger button-response unit (Avotec Inc and Resonance Technologies).

fMRI data acquisition and analysis

Whole-brain functional data were acquired on a GE 1.5-T scanner in 24 axial slices (3.5 × 3.5 × 4.5 mm voxels) parallel to the anterior commissure–posterior commissure (AC–PC) line with a T2*-weighted spiral in-out sequence developed by Dr. Gary Glover [repetition time (TR) = 2000 ms, echo time (TE) = 40 ms, flip angle = 84°, field of view (FOV) = 22 cm]. Structural data were acquired with a T1-weighted spoiled gradient-recalled sequence (1 × 1 × 1 mm; TR = 19 ms, TE = 5 ms, flip angle = 20°).

Functional scans were preprocessed with SPM5, using slice-time correction, motion correction, spatial normalization to the MNI (Montreal Neurological Institute) space and spatial smoothing using a 6-mm full-width at half-maximum Gaussian kernel. Spatial normalization was performed by first co-registering the T1 spoiled gradient recalled (SPGR) to the mean functional image, normalizing the T1 to the SPM template using the 'unified segmentation' algorithm applying the normalization parameters to the functional images and sampling the resulting images at 3 × 3 × 3 mm resolution.

Statistical analyses were conducted using the general linear model framework implemented in Brain Voyager. Boxcar regressors, convolved with the canonical hemodynamic response function, modeled the first 3000 ms of the photoperiod (i.e. excluding the remaining 2000 ms during affect rating). The fixation-cross epoch was used as an implicit baseline. Voxelwise statistical parametric maps summarizing differences between trial types were calculated for each participant and then entered into random-effects group analyses, with statistical maps thresholded for cluster extent at $P < 0.05$ familywise error (FWER) corrected for multiple comparisons across gray and white matter. This correction entailed a primary threshold of $P < 0.01$, with an extent threshold of 29 voxels, which was determined using a Monte Carlo simulation method as calculated using NeuroElf's (http://neuroelf.net/) instantiation of AlphaSim (Forman *et al.*, 1995). This technique controls for the FWER by simulating null datasets with the same spatial autocorrelation found in the residual images and creates a frequency distribution of different cluster sizes. Clusters larger than the minimum size corresponding to the a priori chosen FWER are then retained for additional analysis. This cluster-based method of thresholding is often more sensitive to activation when one can reasonably expect multiple, contiguous, activated voxels (Forman *et al.*, 1995; Petersson *et al.*, 1999) and is widely used in fMRI research. Each main effect was regressed on the mean of contrast values across subjects. Other difference and correlation effects were orthogonal to the mean. The reported activity cluster was found using an FWE-corrected whole brain search. Other statistics are included for the sake of full disclosure. To ensure that the variance of the PTSS measures observed at each time point would have equal weight in the regression with the brain data, the mean for each time point was weighted by its variance prior to averaging. Note that using a simple composite score that averaged across the three time points without transforming the scores did not substantively alter the results.

RESULTS

Individual differences

As Figure 1 illustrates, PTSS scores were significantly related to the 5-HTT genotype. The 9/11 survivors carrying the low expressive short allele ($n = 11$) were characterized by significantly higher levels of PTSS than 9/11 survivors with the long homozygote version of the allele ($n = 6$), $t(16) = 3.45$, $P = 0.003$, $d = 1.73$.

Self-reported emotional reactivity

We performed a repeated measure analysis of variance (ANOVA) with stimulus type (9/11, negative, neutral) as the within-participants factor to examine how self-reported emotional reactivity varied in response to viewing the different stimuli during scanning. This analysis revealed a significant effect of stimulus type, $F(2,14) = 141.29$, $P < 0.001$, $\eta_p^2 = .91$, indicating that participants felt more distressed when viewing 9/11 images compared with both non-9/11 negative images, $t(15) = 6.06$, $P < 0.001$, $d = 3.13$, and neutral images, $t(15) = 12.60$, $P < 0.001$, $d = 6.51$. Participants also experienced more distress when viewing non-9/11 negative images compared with neutral images, $t(15) = 12.01$, $P < 0.001$, $d = 6.20$. Neither PTSS nor genetic polymorphism correlated significantly with self-reported distress ($rs < .38$, $Ps > 0.15$).

Neuroimaging results

Our main predictions concerned the relationship between individual differences in PTSS and the 5-HTT genotype and neural reactivity in

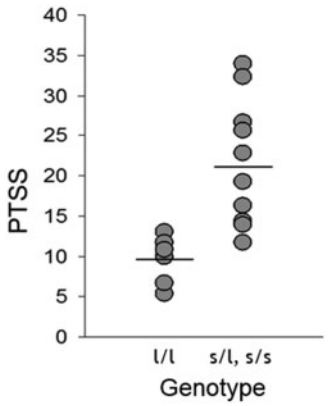

Fig. 1 PTSS as a function of 5HTT genotype; long allele homozygotes (l/l) *vs* carriers of a short allele (s/s and s/l). The horizontal lines represent the group PTSS means.

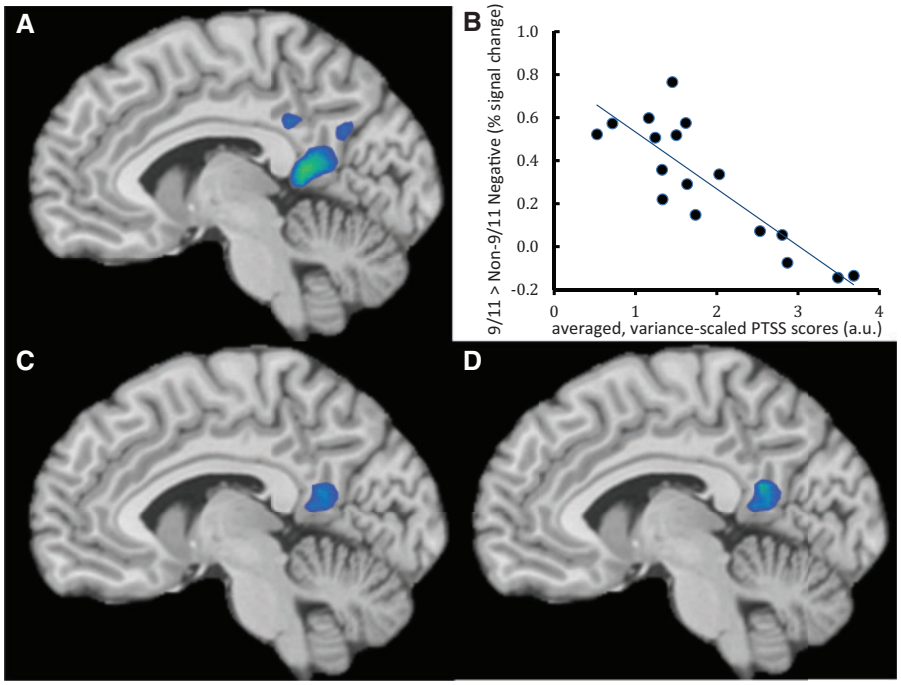

Fig. 2 Brain activity during viewing 9/11 *vs* non-9/11 negative images as related to the averaged, variance-scaled PTSS scores (a.u., arbitrary units) illustrated by (A) its localization, and (B) the correlation between the two variables, where each point represents a single participant. Brain activity during viewing 9/11 *vs* non-9/11 negative images as related to (C) carrying a short allele in the 5-HTT genotype, and (D) both PTSS scores and carrying a short allele (conjunction). Note that these maps show activity at $P < 0.02$ (FWE-corrected using AlphaSim).

response to viewing 9/11 images. We examined these relationships by performing a series of multiple regression analyses to investigate how these individual differences predicted neural activity on 9/11 image *vs* non-9/11 negative image trials. We focused on this contrast because the 9/11 images were matched in terms of valence and were similar in theme (catastrophes) to the non-9/11 negative images. Therefore, comparing negative 9/11 images with negative non-9/11 images provided the most appropriate control when testing our predictions that 9/11 survivors should show less activity in regions related to emotional regulation and self-referential processing, and heightened activity in regions related to emotion generation, in response to trauma-related content[3].

We first examined how PTSS scores correlated with neural activity to 9/11 *vs* non-9/11 negative stimuli. As predicted, this analysis revealed a number of significant negative associations between PTSS and activity in cortical midline regions implicated in self-referential processing including the retrosplenial cortex, and more dorsal regions of the PCC, as well as the precuneus (see Figure 2A and B; for activations see Table 2). We also observed notable significant negative associations between PTSS and activity in caudate and parahippocampal gyrus, which support memory encoding and retrieval processes (Schacter *et al.*, 2007).

Next, we examined the relationship between 5-HTT genotype and neural activity. Participants carrying the low-expressive short allele displayed less activity in a similar network of regions including the PCC, precuneus and parahippocampal gyrus (see Figure 2C; for activations see Table 2).

To examine regions of overlap between the 5-HTT genotype–brain and PTSS–brain correlations we performed a conjunction analysis on the two maps (see Figure 2, Panel D; for activations see Table 2). These analyses revealed activation in a single region of the PCC, including the retrosplenal cortex, which prior research has implicated in PTSD (Liberzon *et al.*, 1999; Pissiota *et al.*, 2002; Lanius *et al.*, 2011).

This finding motivated our final analysis, which explored whether activity in this region of the PCC mediated the relationship between the 5-HTT genotype and PTSS scores.[4] Mediation analyses test whether the relationship between any two variables (here, 5-HTT genotype and PTSS) can be explained by the values from a third variable (activation in the PCC). If PCC activity mediates the genotype–PTSS relationship, then the relationship between these variables should be reduced when PCC is controlled for in the model. Our analysis supported partial mediation, confidence interval (CI) = 1.25–14.32, Sobels $Z = 2.34$, $P < .02$ (see Figure 3). Specifically, genotype was related to PCC activity and PTSS, and the relationship between PCC activity and PTSS was significant when controlling for genotype. As is customary, we used a bootstrapping test (Preacher and Hayes, 2004) to confirm the significant indirect effect of genotype on PTSS via PCC activity, CI = 1.19–15.63 for a 95% CI.

DISCUSSION

The search to understand how genes and brain interact to influence behavior is a central goal of affective science research (Hyde *et al.*, 2011). We addressed this issue by exploring the relationship between neural and genetic markers of vulnerability in a small, but rare sample of 9/11 survivors whose PTSS symptoms were closely tracked over the years following the attack. This sample provided a unique opportunity

[3] The neutral images were primarily selected to give our participants some relief from the intensive exposure to negative images and to prevent habituation. The contrast between 9/11 and Nneutral images did not yield a significant effect in the PCC conjunction cluster, even at a liberal threshold of $P < 0.05$ uncorrected.

[4] Our PTSS score was composed of assessements across three time-points, including two occasions before and one occasion after the measurement of the mediating variable (brain activity). Whereas the outcome variable in most mediation analyses is measured after the mediating variable, we used of a composite PTSS score in this study (rather than the single post- fMRI assessment) to enhance the reliability of the measure, as scores on our three PTSS assessments were highly correlated ($\alpha = .86$) between PTSS scores. Symptom change over the three PTSS assessments did not significantly alter out results, even at an uncontrolled alpha level of $P < 0.05$, for all voxels within the cluster we report in the articlemanuscript.

Table 2 Brain activity during viewing of images related to the 9/11 attack on the World Trade Center and negative non-9/11 related images (Neg)

Region	Laterality	B.A.	Coordinates			T-score	Volume (vox)
			x	y	z		
9/11 > Neg related to PTSS, $P < .01$							
Cingulate gyrus	LH	31	0	−40	31	−8,039	416
Cingulate gyrus	RH	31	13	−41	30	−6,512	
Posterior cingulate	LH	29	−6	−49	11	−6,054	
Posterior cingulate	RH	31	16	−64	16	−5,661	
Precuneus	RH	31	22	−68	25	−5,521	
Posterior cingulate	RH	31	12	−51	20	−5,437	
Precuneus	LH	7	−13	−62	39	−5,295	
Posterior cingulate	LH	23	0	−36	23	−5,217	
9/11 > Neg related to5HTT, $P < .01$							
Posterior cingulate	LH	30	−6	−53	18	−4,095	29
9/11 > Neg conjunction of 5HTT and PTSS, $P < .05$							
Posterior cingulate	LH	30	−6	−53	16	−3,774	317
Precuneus	LH	31	−22	−63	22	−3,3	
Middle temporal gyrus	LH	19	−31	−57	19	−3,201	
Middle temporal gyrus	LH	39	−48	−69	21	−3,2	
Superior occipital gyrus	LH	19	−31	−71	21	−3,108	
Precuneus	LH	31	−19	−74	26	−3,044	
Posterior cingulate	LH	30	−19	−52	9	−2,965	
Middle temporal gyrus	LH	19	−40	−62	14	−2,945	
Middle temporal gyrus	LH	39	−40	−65	24	−2,814	
Precuneus	LH	31	−3	−67	21	−2,617	
Insula	LH	13	−28	−44	22	−2,454	

Note. All *P*'s FWE-corrected for multiple comparisons. BA, Brodmann Area; 5HTT, 5-hydroxytryptamine (serotonin) transporter; PTSS, Post-traumatic stress symptomatology. Each voxel = 3 × 3 × 3 mm.

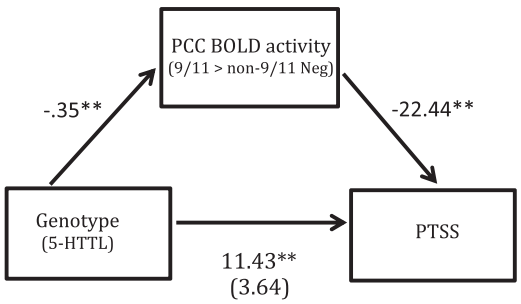

Fig. 3 Standardized regression coefficients for the relationship between genotype (5HTT) and PTS) as partially mediated by blood-oxygen-level dependent (BOLD) activity in the posterior cingulate cortex (PCC). The non-significant coefficient between genes and PTSS when controlling for BOLD activity in PCC is in parentheses. $^{**}P \leq 0.01$.

to examine the psychological and biological bases of responses to a traumatic event of national importance. Three key findings emerged.

First, we found that 9/11 survivors who were carriers of the low-expressive short allele in the promoter region of the 5-HTT gene reported higher level of PTSS. This observation conceptually replicates prior research, indicating that people who possess this gene variant are more likely to develop PTSD after exposure to traumatic events (Grabe *et al.*, 2009; Koenen *et al.*, 2009).

Second, both PTSS and the presence of the short allele predicted reduced levels of activity in several cortical midline regions that support self-referential processing and episodic memory, including the PCC (Raichle *et al.*, 2001; Buckner *et al.*, 2008). To the extent that people with PTSD habitually experience numbness and avoid focusing on their negative past experiences, one would expect symptoms of PTSS to correlate inversely with activation in these regions. The negative associations we observed between midline regions and PTSS and 5-HTT were consistent with this view. So was the decreased activation we observed in the parahippocampal region to trauma-related when compared with negative control images—a finding that dovetails with previous findings indicating that PTSD patients, when compared with normal controls, show decreased activity (Werner *et al.*, 2009) and volume (Papagni *et al.*, 2011; Liu *et al.*, 2012) in this region, which is known to be involved in the retrieval of episodic memories (Schacter *et al.*, 2007). A recent study (Whalley *et al.*, 2013) in PTSD patients found deactivations in the PCC, precuneus and parahippocampal regions during the retrieval of flashback-type memories. The authors reasoned that these deactivations might reflect the decontextualization of sensory representations or even efforts to terminate self-reflection associated with the trauma memories, which would be consistent with our results. This interpretation would also be compatible with the fact that a decrease in activity in the midline regions is typically observed when attention is located to external stimuli or tasks, such as when trying to distract oneself (Burgess *et al.*, 2001; Leech and Sharp, 2014).

Finally, these two findings motivated exploratory analyses indicating that PCC activity mediated the relationship between genotype and PTSS. Although these results are preliminary, they highlight a hithertho unexplored potential neural pathway through which genes, in particular 5-HTT, may influence the development and expression of PTSS. Activity in the PCC is a relatively unexplored potential biomarker in PTSD. Because of its close link to genetic makeup in our sample, our findings suggest that PCC may play a role as an endophenotype for PTSD.

Three caveats are in order before concluding. First, it is important to recognize that our study did not include a healthy control group, which constrains direct comparisons of our results with prior studies that have included such a comparison. For example, whereas some studies (Etkin and Wager, 2007) have reported hyperactivity in the amygdala (although inconsistently so, see for example Britton *et al.*, 2005; Phan *et al.*, 2006), insular and corticomidline (Sartory *et al.*, 2013) regions in PTSD patients *vs* healthy controls, we did not observe elevated activity in these regions in response to 9/11-related images in our sample. That said, a recent meta-analysis shows that hyperactivations of the amygdala and insular region are less characteristic of patients with PTSD when compared with other anxiety disorders (Etkin and Wager, 2007), suggesting that deficits in emotion regulation and self-reflection play an important role in PTSD.

Second, we used a rare group of 9/11 survivors characterized by a high degree of homogeneity in terms of timing and experiences of the trauma. Unfortunately, this limited the number of available participants. In acknowledgement of this limitation, our main predictions were confirmatory and were followed up by an exploratory mediation analysis. We believe that this approach and the strong effects obtained together with the uniqueness of the sample jointly underscore the importance of our results.

Finally, we did not assess the rare presence of a single nucleotide polymorphism within the promoter region of the 5-HTTLPR (rs25531), which has been shown to modulate the transcriptional efficiency of the 5-HT transporter (Murphy and Lesch, 2008). We did not assess this polymorphism because no commercially available assay for rs25531 was available at the time of this study.

In sum, activation of a midline cortical region known for its engagement in autobiographical memory retrieval and self-referential processing correlated 'negatively' with genetic indices of vulnerability when high-exposure 9/11 survivors were shown 9/11-related images 6 years after the trauma. These findings add to existing imaging studies showing decreased midline activation in PTSD and extensive behavioral research demonstrating a strong positive relationship between avoidance and PTSD. Our results also highlight the relationship

between PTSS and potential biomarkers of such dysfunctional responses to trauma, such as activity in the PCC. Because this activity was tightly linked to a common genetic polymorphism, it might serve as a candidate endophenotype of PTSD to be explored in future research.

Conflict of Interest

None declared.

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