Archival Report

Biological Psychiatry

Dorsomedial Prefrontal Cortex Mediates the Impact of Serotonin Transporter Linked Polymorphic Region Genotype on Anticipatory Threat Reactions

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ABSTRACT

BACKGROUND: Excessive anticipatory reactions to potential future adversity are observed across a range of anxiety disorders, but the neurogenetic mechanisms driving interindividual differences are largely unknown. We aimed to discover and validate a gene-brain-behavior pathway by linking presumed genetic risk for anxiety-related psychopathology, key neural activity involved in anxious anticipation, and resulting aversive emotional states.

METHODS: The functional neuroanatomy of aversive anticipation was probed through functional magnetic resonance imaging in two independent samples of healthy subjects (n = 99 and n = 69), and we studied the influence of genetic variance in the serotonin transporter linked polymorphic region (5-HTTLPR). Skin conductance and startle data served as objective psychophysiological indices of the intensity of individuals' anticipatory responses to potential threat.

RESULTS: Threat cues signaling risk of future electrical shock activated the dorsomedial prefrontal cortex (dmPFC), anterior insula, bed nucleus of the stria terminalis, thalamus, and midbrain consistently across both samples. Threat-related dmPFC activation was enhanced in 5-HTTLPR short allele carriers in sample 1 and this effect was validated in sample 2. Critically, we show that this region mediates the increase in anticipatory psychophysiological reactions in short allele carriers indexed by skin conductance (experiment 1) and startle reactions (experiment 2).

CONCLUSIONS: The converging results from these experiments demonstrate that innate 5-HTTLPR linked variation in dmPFC activity predicts psychophysiological responsivity to pending threats. Our results reveal a neurogenetic pathway mediating interindividual variability in anticipatory responses to threat and yield a novel mechanistic account for previously reported associations between genetic variability in serotonin transporter function and stress-related psychopathology.

Keywords: Anxiety, Fear, fMRI, 5-HTTLPR, Psychophysiology, Serotonin transporter

http://dx.doi.org/10.1016/j.biopsych.2014.07.034

Approximately 30% to 40% of individual differences in anxiety are determined by genetic predisposition (1). However, the underlying neurobiological mechanisms remain to be discovered. Identifying biological cascades through which genetic risk factors instantiate anxiety via their impact on brain function can shed light on the pathophysiology of anxiety disorders, the most prevailing form of psychiatric disease (2). Critically, the practical use of such knowledge requires validation in independent experiments to show that results generalize (3).

The serotonin transporter gene (*SLC6A4/5-HTT/SERT*) is an important candidate gene for anxiety research, given that this transporter is an efficient pharmacologic target for treatment of a range of anxiety-related disorders (4). A common polymorphism in the promoter region of this gene (serotonin transporter linked polymorphic region [5-HTTLPR]) moderates *SERT* expression levels, with the 5-HTTLPR short (S) allele being linked to lower gene expression (5). One prominent hypothesis is that heightened anxiety in 5-HTTLPR short allele carriers (S-carriers) (6,7), as well as the reported risk to develop stress-related mental disorders, emerges out of an increased responsiveness to stressors (8,9). Enhanced amygdala responses in S-carriers, when presented with threatening stimuli, might be one mechanism (10,11). However, in addition to reactions to directly aversive stimuli, anticipatory processes that are initiated in the context of potential future adversity have been suggested to be an important focus for research into the fundaments of anxiety-related disease (12,13).

The anticipation of potential threat in the future is consistently associated with activity in a neural salience network that prominently includes the dorsomedial prefrontal cortex (dmPFC), anterior insula, and midbrain (12,14–16). Specifically,

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 ISSN: 0006-3223

a convergence of work in animals and humans now implicates the dmPFC in the expression of aversive states while anticipating adversity (17,18). Recent studies provide initial evidence that S-carriers show enhanced reactions within several regions of the salience network including the dmPFC (19–21); however, to date, no studies have directly validated such gene-brain associations in independent participant samples. Moreover, it remains to be established how innate variance in neural activity would effectively mediate enhanced anxiety in S-carriers as indexed by objective psychophysiological responses to threat (22,23).

In the current study, we aimed to uncover a neurogenetic mechanism underlying individual differences in objective anxiety measures by studying neural and psychophysiological anticipatory responses to threat and their relation to the 5-HTTLPR polymorphism. To test whether identified genetic association would generalize across different experimental setups and study samples, we report results here from two independently designed experiments. Healthy participants, without potential disease-related confounds such as medication use, were genotyped for the 5-HTTLPR polymorphism and subjected to either a classical fear conditioning (experiment 1) or instructed fear (experiment 2) procedure. Functional magnetic resonance imaging (fMRI) measures were supplemented by two commonly used objective psychophysiological measures of aversive states: skin conductance responses in experiment 1 and eye-blink startle reflexes in experiment 2.

METHODS AND MATERIALS

Studies were approved by local medical ethical committees from Radboud University Medical Centre and University Medical Centre Utrecht (UMCU), respectively.

Subjects

Participants were recruited through advertisements posted around the Radboud University Medical Centre (sample 1: 99 male subjects) and UMCU (sample 2: 69 subjects, 21 male subjects). All subjects were aged 18 to 30 and reported no regular use of psychoactive drugs or history of neurologic and psychiatric disorders. Sample 2 consisted of subjects described in previous publications for whom fMRI data were not previously analyzed as a function of genotype. More specific, for the current study, we genotyped 21 subjects from a sample that was previously scanned without the aim of genetic analysis (14). An additional 48 subjects for whom genotype effects on startle data were described earlier were scanned to further increase sample size (22). From this latter study, we scanned as many homozygotes as possible (for both short and long allele) to enhance statistical power for genetic comparisons. More than 90% of participants in each sample were of North European origin. After a complete description of the study to the subjects, written informed consent was obtained.

Genotyping

For sample 1, DNA was isolated from saliva using Oragene containers (DNA Genotek, Kanata, Ontario, Canada). For sample 2, DNA was collected with buccal swabs and isolated using a standardized kit (QiAmp DNA Mini Kit; Qiagen, Hilden, Germany). 5-HTTLPR genotyping was performed using polymerase chain reaction followed by sequence length analysis using an automated capillary sequencer (ABI3730, Applied Biosystems Foster City, California; sample 1) or standard gel electrophoresis (sample 2) to classify each subject as having either two short 486 base pair DNA fragments (S/S), one short and one long (529 base pair) fragment (S/L), or two copies of the long fragment (L/L). Detailed information on primers and procedures is available from the authors upon request. Genotype distributions can be found in Table 1. In line with previous research (3,19,21,22), in all analyses, short allele carriers (S/S and S/L) were contrasted statistically with LL homozygotes. Yet, for illustrative purposes, data for each genotype group (SS, SL, LL) are always displayed separately, as recommended (3).

Experimental Designs

Experiment 1. Subjects of sample 1 were informed that they would see a yellow or blue square on a computer screen and that electrical shocks would be administered. The level of the shocks, administered to the fingers, was set before the experiment to a subjective intensity that was maximally uncomfortable without being painful to the subject. Subjects were instructed to pay attention to the screen and were informed that a relationship existed between the stimuli and shocks. Colored squares were presented for 4 seconds in pseudorandomized order. Each stimulus was presented 18 times with an intertrial interval of 11 to 13 seconds. One square color co-terminated with the presentation of the electric shock stimulation on one third of the trials; the other color was never paired with electric stimulation. Given the slow nature of skin conductance responses (SCRs), only no-shock trials were used for analyses to exclude reactions to the shocks.

Table 1. Sample Size Expressed as Number of Subjects and as Percentage for Each Sample, Including Sex Distribution, Age, and Trait Anxiety Scores (Mean, SD) as a Function of 5-HTTLPR Genotype

	Sample 1 (n = 99)			Sample 2 (n = 69)		
	S/S	S/L	L/L	S/S	S/L	L/L
n (% of Sample)	16 (16%)	53 (54%)	30 (30%)	15 (22%)	22 (32%)	32 (46%)
% Male Subjects	100	100	100	27	32	44
Age	21.9 (2.3)	22.0 (2.6)	21.8 (2.7)	22.0 (2.1)	21.9 (2.8)	21.6 (2.2)
Trait Anxiety STAI-T	39 (8.5)	35.6 (7.6)	35.7 (6.9)	33.5 (8.8)	35.2 (8.6)	31.5 (7.5)

5-HTTLPR, serotonin transporter linked polymorphic region; L, long; S, short; STAI-T, trait portion of the Spielberger Trait Anxiety Inventory.

Experiment 2. Subjects of sample 2 were explicitly informed beforehand that during presentation of one particular picture of a male face with neutral facial expression, shocks might be administered at any time (14). A second male face with neutral facial expression was instructed to never be associated with shocks. Subjects were instructed to rest when there was no cue on the screen and accordingly the word REST (in Dutch) was presented during the intertrial interval. Again, each subject underwent a standardized procedure to set shock intensities individually before the experiment (14). The experiment consisted of 42 presentations of each cue in semi-random order with cue durations jittered between 6 and 12 seconds and an intertrial interval of 8 to 12 seconds. A shock was administered at unpredictable times during one out of every six threat cues.

Imaging

Experiment 1. Magnetic resonance (MR) data of Experiment 1 were acquired on a 1.5 T Avanto MR scanner (Siemens, Erlangen, Germany) at the Donders Institute in Nijmegen. A series of 302 T2*-weighted functional images were acquired using gradient echo-planar imaging with the following parameters: 32 oblique transverse slices, voxel size = 3.5 × 3.3 × 3.3 mm, repetition time (TR) = 2.34 seconds, flip angle $\alpha = 90^{\circ}$, echo time (TE) = 35 milliseconds. A three-dimensional (3-D) magnetization prepared rapid acquisition gradient-echo anatomical T1-weighted image was acquired for normalization purposes (176 slices, 1.0 mm isotropic, TR = 2730 msec, TE = 2.95 msec).

Experiment 2. Imaging of experiment 2 was performed on a Philips 3T Achieva MRI scanner (Philips Medical Systems, Best, The Netherlands) at UMCU. In three runs, 2250 T2*-weighted volumes were acquired using a 3-D principle of echo shifting with a train of observations (PRESTO) sequence (39 sagittal slices, voxel size = 3.5 mm isotropic, TR = .813 msec, $\alpha = 8.85^{\circ}$, TE = 23 msec). A T1-weighted anatomical image was again obtained for normalization (175 sagittal slices, 1 mm isotropic, TR = 8.4 msec, $\alpha = 17^{\circ}$, TE = 3.8 msec).

Psychophysiological Recordings

Experiment 1. Electrodermal activity was assessed during scanning with silver/silver chloride electrodes attached to the subject's distal phalanges of the index and middle finger of the nondominant hand. The skin conductance signal was amplified using the BrainAmp MR system and recorded using BrainVision Recorder software (Brain Products GmbH, Munich, Germany). Skin conductance data were assessed using an in-house analysis program written in MATLAB (MathWorks, Natick, Massachusetts) and using FieldTrip. Data were low-pass filtered at 5 Hz. Skin conductance responses were determined for each trial as the peak-to-peak amplitude difference in skin conductance of the largest deflection in the latency window from 0 to 8 seconds after stimulus onset. All results, including the genotype effects, were confirmed in an analysis on peaks from 0 to 4 seconds following stimulus onset. These responses were subsequently square root transformed in accordance with previous literature (18).

Experiment 2. For experiment 2, startle reflex intensity was recorded outside the MR scanner in a session separated from the MRI session by 5 to 21 days. The same paradigm was used for both sessions with session order counterbalanced across participants. To induce startle responses, auditory startle probes consisting of 50-msec white noise bursts were presented during the cues, 1.5 to 11.5 seconds after cue onset, at 106 dBa through foam in-ear earplugs (Earlink; Aearo Company Auditory Systems, Indianapolis, Indiana). Electromyographic recording and amplification of the eyeblink startle reflex was carried out using the BioSemi Active Two system with matching FLAT active silver/silver chloride electrodes (BioSemi, Amsterdam, The Netherlands). Recordings were made from the orbicularis oculi sphincter muscle by centralizing one electrode under the right pupil and the other 15 mm lateral toward the outer canthus of the eye. Data were preprocessed and checked for artifacts according to published guidelines (24) and procedures (14) using Brain Vision Analyzer (Brain Products GmbH) and custom-made MATLAB software. In short, the signal was baseline corrected, rectified, and smoothed using a 16 Hz low-pass filter. Startle amplitudes were subsequently quantified as the highest peak between 25 and 100 milliseconds after probe onset. Finally, startle magnitudes were transformed to T scores per subject (24).

fMRI Data Analysis

Functional scans from both experiments were realigned and subsequently co-registered to the anatomical scan to spatially normalize functional images via the anatomical scan to the Montreal Neurological Institute 152 T1-template image via the unified segmentation procedure in SPM8 (Wellcome Trust Centre for Neuroimaging, London, United Kingdom; http://www.fil.ion.ucl.ac.uk/spm/). The normalized images (3.5 mm isotropic for both experiments) were then smoothed with an isotropic 3-D Gaussian kernel with a full-width at half maximum of 8 mm. In SPM, general linear models were composed to relate blood oxygen level-dependent (BOLD) signal variation in each voxel to the task conditions. The predictors of neural activity were the threat conditions, safe conditions, and shocks and these were modeled with boxcars with appropriate durations. Following previous work (14), in experiment 2, both the onset and offset responses to each cue were additionally modeled using a delta function (zero duration; analyses on the offset responses will be reported elsewhere). All regressors were convolved with the canonical hemodynamic response function in SPM. Realignment parameters were included in the model as regressors of no interest. High-pass filtering (cutoff 128 seconds) and a first-order autoregressive model were used as standard in SPM. Reactions to threat and safe cues were contrasted in each subject to index threat-related responses. For experiment 1, this entailed contrasting responses modeled by the 4-second boxcars. For experiment 2, threat onsets were compared with those of the safe cue. Analyses of the 6-second to 12-second boxcar regressors in experiment 2 revealed very similar threat-responsive regions as reported below for the onset regressors [cf. (14)]; they are omitted for brevity, as the onset regressors are arguably more comparable with the data from experiment 1 given the longer

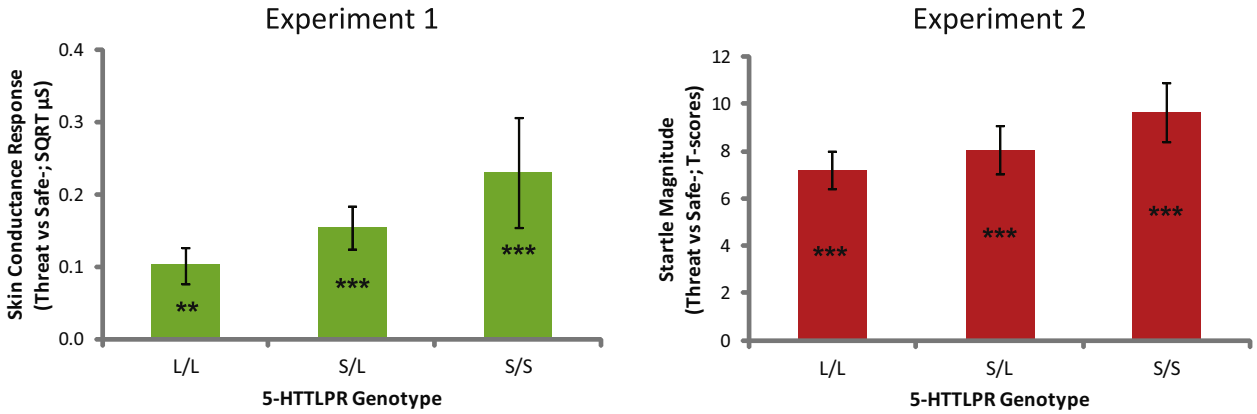

Figure 1. Mean psychophysiological responses to threat as a function of serotonin transporter linked polymorphic region (5-HTTLPR) genotype. The left panel shows skin conductance data from sample 1 (n = 99) and the right panel shows startle data from sample 2 (n = 69). Significant increases in threat-related responding relative to safe cues were observed in all groups. Genotype effects did not reach significance in each sample separately but a post hoc analysis across the two samples indicated a significant impact of the 5-HTTLPR polymorphism. Asterisks indicate significance of threat versus safe contrast. $^{**}p < .01$; $^{***}p < .001$. L, long; S, short; SQRT, square root.

cue durations in experiment 2. The single subject contrast maps were subsequently subjected to random effects analyses. Whole-brain results were thresholded at $p < .001$ uncorrected combined with a cluster threshold of $p < .05$ family-wise error corrected for multiple comparisons according to random field theory implemented in SPM. Next, to attempt to validate in sample 2 the significant genotype effects in our regions of interest (ROI) observed in the larger sample (sample 1), we extracted the mean beta weight for sample 2 specifically from clusters within our core regions of interest (dmPFC and insula) that showed a genotype effect in sample 1. In this way, we could test in a single, focused analysis per region whether the effects in sample 1 were validated by the data from the same regions in sample 2. To accommodate potential differences in anatomy and normalization between the samples, we chose to define these clusters of interest using a cluster-defining threshold of .005 uncorrected (similarly significant results were obtained, however, at the original threshold of .001 uncorrected). Finally, to test whether genotype-dependent neural activity might mediate an impact of genotype on psychophysiological responses, we performed a mediation analysis with accelerated bias-corrected bootstrap significance testing (10,000 bootstrap samples) as implemented in the M3 toolbox (http://wagerlab.colorado.edu/tools) (25).

RESULTS

Subject Descriptives

For sample 1, genotype distribution was in accordance with previous studies and in Hardy-Weinberg equilibrium (p = .36). As intended, selection led to a higher proportion of homozygotes in sample 2 (Table 1). There were no significant differences in either experiment between the genotype groups with regard to sex distribution, age, and trait anxiety (all p values > .09; Table 1). Given that subjects in sample 1 were all male subjects, sex was controlled for in the genotype analyses reported below for sample 2.

Psychophysiological Responses to Threat Consistently Scale According to 5-HTTLPR Genotype

Threat cues produced significant increases in psychophysiological responses relative to the safe cues as measured by skin conductance responses in experiment 1 (repeated measures analysis of variance threat vs. safe: $F_{1,98}$ = 59.8, $p < .001$) and fear-potentiated startle in experiment 2 (threat vs. safe: $F_{1,68}$ = 200.8, $p < .001$). Analyses on each experiment separately did not establish significant genotype effects (5-HTTLPR [S-carriers, LL] × threat [threat, safe] interaction; sample 1: p = .13, sample 2: p = .10). However, in each sample, the mean intensity of psychophysiological responses appeared to increase consistently with the number of S-alleles (Figure 1). A post hoc omnibus repeated measures analysis across both samples with experiment as factor did yield a p value < .05 (5-HTTLPR × threat $F_{1,163}$ = 4.9, p = .028). Thus, we evoked robust anticipatory threat-related psychophysiological responses in each experiment and found evidence in a post hoc analysis that these reactions could be modulated by 5-HTTLPR genotype as described in previous work (8–12).

Consistent Anticipatory Neural Responses to Threat

In both samples, we observed that threat was associated with BOLD signal increases in a strongly overlapping neural network encompassing the bilateral dorsal medial prefrontal cortex, anterior insula, ventral striatum/bed nucleus of the stria terminalis (BNST), and thalamus/midbrain (Figure 2), among other areas, but not the amygdala [cf. (14,15)] (full results in Table S1 in Supplement 1). Thus, we found consistent anticipatory neural activation to threat across both experimental setups.

The dmPFC Consistently Mediates the Effect of 5-HTTLPR Genotype on Anticipatory Psychophysiological Responses to Threat

Whole-brain analysis in sample 1 revealed greater activity to threat cues in S-allele carriers than LL homozygotes in the

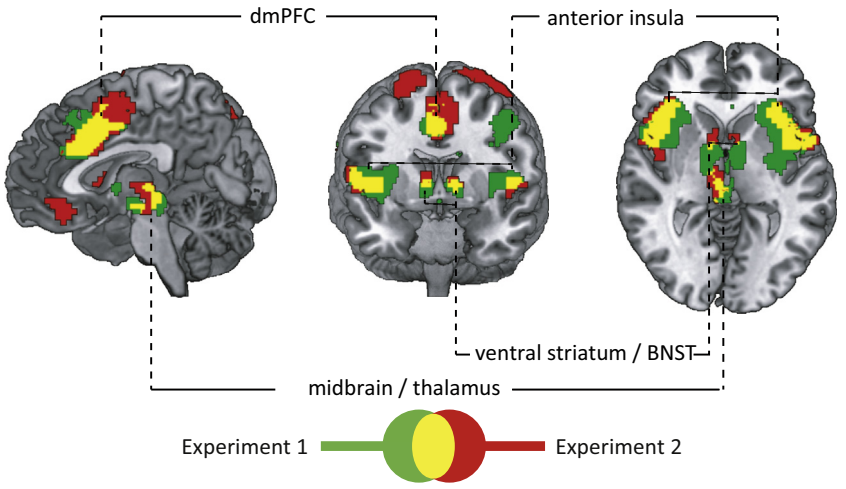

Figure 2. Neural responses to threat across all genotypes. The images show areas that reach an uncorrected voxelwise threshold of $p < .001$ for the contrast threat versus safe in experiment 1 ($n = 99$; in red) and experiment 2 ($n = 69$; in green). Overlap is shown in yellow. All labeled clusters reach cluster-level corrected significance in each sample separately (family wise error corrected at the cluster level corrected $p < .05$). BNST, ventral striatal region overlapping with the bed nucleus of the stria terminalis; dmPFC, dorsomedial prefrontal cortex. Due to the small size of the BNST, this labeling should be taken with caution.

threat-responsive regions in the dmPFC and anterior insula (5-HTTLPR × threat $ps < .05$ family wise error corrected at the cluster level; Figure 3; Table S2 in Supplement 1). Critically, while initial whole-brain analyses did not reveal genotype effects in sample 2 (Table S2 in Supplement 1), a comparable genotype effect in sample 2 was revealed in the mean activity of the dmPFC region that was shown to be genotype sensitive in sample 1 (5-HTTLPR × threat sample 2: $F_{1,69} = 6.16$, $p = .015$; Figure 3). The bilateral insula ROI showed a similar pattern but failed to reach significance. Subsequent exploratory regions of interest analyses on the BNST and amygdala anatomical ROIs did not reveal significant genotype effects. Thus, dmPFC activity was affected by 5-HTTLPR genotype in sample 1 during the anticipation of potential aversive outcome, and we validated this result in the independent data from the corresponding region in sample 2. Interestingly, in both experiments, we found a positive correlation between activity in this genotype-sensitive dmPFC region and psychophysiological reactions to threat (Figure 3, scatter plots). Subjects exhibiting increased threat-related dmPFC responses consistently exhibited elevated anticipatory psychophysiological reactions (sample 1 $Rs = .29$, $p = .003$; sample 2 $Rs = .33$, $p = .005$), suggesting that dmPFC activity might mediate the effect of 5-HTTLPR genotype on psychophysiological reactions.

We subsequently formally tested through hierarchical linear regression analysis (25) whether dmPFC activity mediated the relationship between 5-HTTLPR genotype and psychophysiological reactions to threat. To avoid nonindependence concerns, we extracted dmPFC activity from the ROI that showed a main effect of threat for sample 1 (independent of genotype). Mediation analyses on sample 1 confirmed that S-carriers showed increased dmPFC activity to threat (path *a*: $t_{98} = 3.97$, $p < .001$), greater dmPFC activity predicted greater psychophysiological reactions (path *b*: $t_{97} = 3.50$, $p < .001$), and dmPFC activity significantly mediated 5-HTTLPR effects on psychophysiological reactions (path *ab*: $t_{98} = 2.47$, $p = .009$). We replicated these mediation effects in sample 2, where we again found increased dmPFC activity for S-carriers (path *a*: $t_{69} = 2.24$, $p = .009$), greater dmPFC activity to predict greater psychophysiological responses (path *b*: $t_{68} = 3.01$, $p = .002$), and dmPFC activity to mediate 5-HTTLPR effects on psychophysiological responses (path *ab*: $t68 = 1.69$, $p = .011$) (Figure 4). Thus, threat-related activation in the dmPFC mediated the previously reported relationship between 5-HTTLPR genotype and psychophysiological responses across both samples.

Finally, for a reduced sample (96 subjects in sample 1 and 56 subjects in sample 2), we could additionally take into account another functional polymorphism in the 5-HTT gene (rs25531). Contrasting subjects with purported high (La/La) and low 5-HTT expression (S- and/or Lg-allele carriers) based on this triallelic haplotype (19,20,26), we obtained highly similar results, including the significant mediation effect in both subsamples (Figure S1 in Supplement 1).

DISCUSSION

The identification of neurobiological pathways underlying interindividual differences in psychophysiological reactions during the anticipation of adversity has the exciting outlook of uncovering fundamental mechanisms that might determine vulnerability to anxiety disorders. Here, we validate across two independent samples with different experimental setups that a genetic variation in the 5-HTTLPR predicts psychophysiological reactions to threat mediated by its impact on neural processing in the dmPFC. Our results increase understanding of the mechanisms through which individual differences in anxiety emerge from our innate neurobiological makeup. Moreover, these results provide a novel mechanistic explanation for previously reported 5-HTTLPR-linked genetic predisposition to stress-related mental disease (8).

Significant threat-related increases in skin conductance and startle potentiation responses, established psychophysiological indices of aversive states, confirmed the effectiveness of our experimental procedures. Delineating a neural circuitry of aversive anticipation, we found threat-related activations in a neural salience network consistently for both experimental setups. This finding converges nicely with previous work

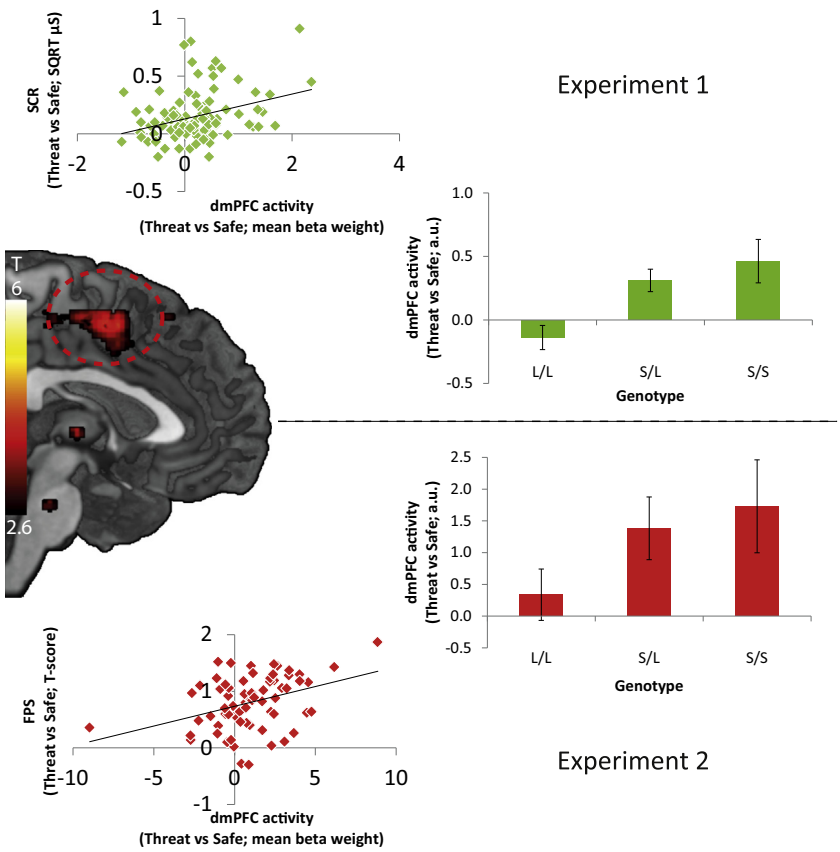

Figure 3. Threat-related activity in the dorsomedial prefrontal cortex (dmPFC) is consistently related to 5-HTTLPR genotype and shows a reliable association with psychophysiological responses. A significant threat-by-5-HTTLPR genotype interaction was observed in the dmPFC in sample 1 (top right bars) and validated in sample 2 (bottom right bars). Neural activity in this region, shown on the left ($p < .005$ cluster defining threshold; FWE_c corrected for multiple comparisons $p < .05$) consistently predicted psychophysiological responding indexed by skin conductance responding (SCR) (top left scatter) and fear-potentiated startle (FPS) (bottom left scatter). Note that the rectangular shape for the upper part of the activation is due to more dorsal brain regions falling just outside the field of view in some subjects with larger brains. While the scatter plots might suggest an influence of outliers, Spearman rank correlations are reported to protect against the impact of extreme values. All testing outcomes remain significant also after removing the apparent outlier values farther than three SD from each sample's mean value. SQRT, square root; 5-HTTLPR, serotonin transporter linked polymorphic region; FEWc, family wise error corrected at the cluster level; S, short alleles; L, long alleles.

(12,15) implicating these regions in the expression of aversive states and stress (17,18). The dmPFC is suggested to drive expression of threat-related defensive responses (18), and more generally the dmPFC has been associated with outcome evaluation under uncertainty. Other regions, such as the anterior insula, midbrain, and BNST, have all also been implicated in the anticipation of aversive stimuli and have been linked more specifically to subjective feeling states and the interoceptive awareness of visceral responses (27,28), defensive reactions such as freezing (29), and the expression of sustained anxiety (30).

Consistent with previous observations (23), psychophysiological responses to threat appeared strongest in carriers of the S-allele for the 5-HTTLPR polymorphism, although we could only statistically demonstrate this effect in a post hoc analysis over both samples. We subsequently sought to investigate the neural mechanism underlying this genetic predisposition for elevated responses to threat. Establishing

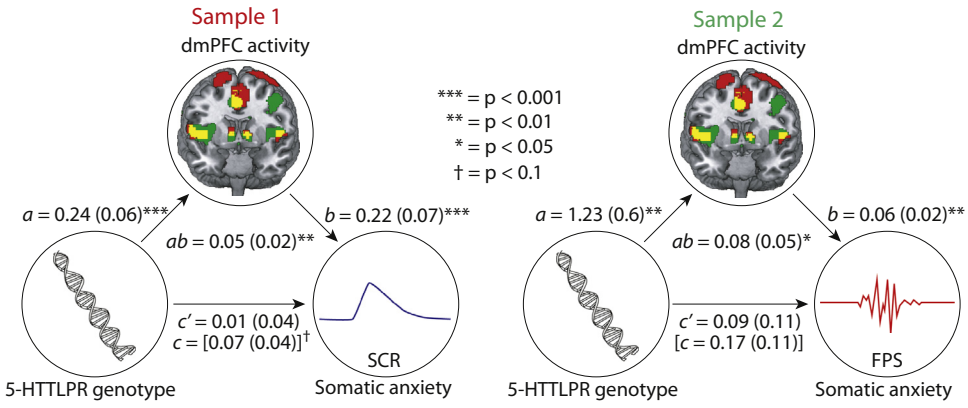

Figure 4. Activation in the dorsomedial prefrontal cortex (dmPFC) mediated the relationship between serotonin transporter linked polymorphic region (5-HTTLPR) genotype and psychophysiological responses to threat in both sample 1 (left) and sample 2 (right). Genotype at 5-HTTLPR predicts threat-related activity in the dmPFC (path *a*). Activity in the dmPFC mediator region predicts the outcome, i.e., skin conductance response (SCR) and fear-potentiated startle (FPS) (path *b*). The direct connection from 5-HTTLPR to psychophysiological responses controlled for the mediation effect is provided at the bottom (path *c*′) and the total effect is in square parenthesis [path *c*]. The lines are labeled with path coefficients, and standard errors are shown in parentheses. Asterisks indicate *p* values (two-tailed).

a potential neural mechanism for enhanced anxiety vulnerability in S-carriers, we observed that S-allele carriers showed greater threat-related BOLD responses in the dmPFC in sample 1 and validated this effect in sample 2. Interestingly, subjects with more threat-related activation in this genotype-sensitive dmPFC region also consistently showed the strongest psychophysiological responses to threat. This suggested that the more distant, statistically less strong effects of 5-HTTLPR genotype on psychophysiological responses might be mediated by a mutual association with the dmPFC. Following up on these findings, we provided direct evidence in both samples that activation in this dmPFC region mediates enhanced anticipatory psychophysiological responses to threat in S-carriers. For the first time, we thereby validate across two samples a gene-brain-behavior association underlying stronger psychophysiological reactivity to threat in 5-HTTLPR S-carriers (3,22,23).

Our findings reveal a mechanism centered on the dmPFC by which 5-HTTLPR genotype affects aversive anticipatory states. Preceding work suggested that stronger amygdala reactivity to emotional stimuli, such as aversive pictures or fearful faces (10,11), might underlie vulnerability to develop anxiety disorders in 5-HTTLPR S-carriers (6,31). The dmPFC has strong connections to the amygdala, as well as to midbrain regions that have been shown to play important roles in psychophysiological responses to stress (17). There are no indications in the current literature for 5-HTTLPR genotype effects on *SERT* expression in the dmPFC (32), yet the dmPFC shows a relatively high serotonin transporter density, as do the amygdala and midbrain (33). Given that dmPFC hyperactivity is implicated in the resistance to extinction learning (34,35), neuromodulatory treatments targeting dmPFC hyperactivity might ameliorate anxiety symptoms (36), and our results suggest this might be particularly important in those with innate hyperreactivity of the dmPFC.

It has to be emphasized that an innate tendency to show heightened anticipatory physiological responses during possible threats is not necessarily maladaptive. Recent work even suggests that S-carriers adapt more rapidly to a changing environment and hence exhibit faster learning (37,38). Further, heightened vulnerability for psychiatric disorders in S-carriers has been shown to be conditional on exposure to severe stress (8,9). Both acute and chronic stress give rise to profound alterations in serotonin levels (39,40), and interaction with serotonergic genetics might increase the risk for developing disease (41). Furthermore, here, we focused on one well-described polymorphism that forms a minor part of the total relevant genetic background for anxiety. Clearly, numerous genetic variants contribute, including others affecting serotonin transporter expression (26,42). Taking into account additional functional genetic variation, for example, by sampling a larger portion of the variance across genes within the serotonergic pathway (43), together with the assessment of critical life events (8,21), could be used in future studies to increase power to predict anxiety on the individual level and, ultimately, select optimal treatment strategies for patients. Our results suggest that assessment of dmPFC function may then serve as a marker closer to objective anxiety levels than genetic markers.

In conclusion, we delineated and validated a previously unknown neurogenetic mechanism underlying individual differences in the expression of anticipatory anxiety, a core symptom in anxiety disorders. We demonstrated that the 5-HTTLPR affects the intensity of aversive anticipatory states through its effects on neural activity in the dmPFC. Our results provide a new mechanistic explanation for previously reported associations between genetic variability in serotonin function and a stress sensitive phenotype.

ACKNOWLEDGMENTS AND DISCLOSURES

FK, MK, JLK, GvW, GF, and JMPB designed the experiments. FK and MK performed data analyses. BF, IH, RSO, and FK were responsible for genetic analyses. FK, MK, GF, and JMPB wrote the article. All authors commented on previous versions of the manuscript and agreed upon the final version of the manuscript.

This work was supported by funding from the Dutch organization for scientific research (NWO) through grants awarded to GF (VIDI grant) and GvW (VENI grant). We acknowledge technical assistance and contributions to data acquisition by Sabine Kooijman, Nicole Driessen, Yuen Fang, Veerle van Son, and Anita Harteveld.

All authors report no biomedical financial interests or potential conflicts of interest.

ARTICLE INFORMATION

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Received Mar 8, 2014; revised Jul 18, 2014; accepted Jul 25, 2014.

Supplementary material cited in this article is available online at http://dx.doi.org/10.1016/j.biopsych.2014.07.034.

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