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Interactive effect of 5-HTTLPR and BDNF polymorphisms on amygdala intrinsic functional connectivity and anxiety

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ABSTRACT

The serotonin transporter (5-HTTLPR) and brain-derived neurotrophic factor (*BDNF*) gene polymorphisms have been associated with risk for affective disorders and functional variability of the amygdala. We examined whether the two genotypes interactively influence intrinsic functional connectivity (FC) of the amygdala and whether FC mediates the genetic association with anxiety. Eighty genotyped healthy adults underwent resting state fMRI and completed the self-reported State-Trait Anxiety Inventory. Interactive genetic association with anxiety was observed such that effects of 5-HTTLPR depended on the *BDNF Val66Met* polymorphism (rs6265 variant), with higher anxiety scores in short and Met carriers compared to the other allelic groups. Voxel-wise FC with left and right amygdala seeds identified regions that were sensitive to variability in anxiety scores. A significant moderated mediation model demonstrated that the effect of 5-HTTLPR genotype on anxiety, moderated by *BDNF Val66Met* genotype, was fully mediated by FC between the left amygdala and the right dorsolateral prefrontal cortex, a cognitive control-related region, during a task-free state. FC was highest in carriers of the 5-HTTLPR short allele and *BDNF* Met allele. These findings establish intrinsic amygdala-prefrontal functional connectivity as a potential intermediate phenotype for anxiety, an important step toward identification of causal pathways for vulnerability to affective disorders.

1. Introduction

In delineating causal pathways of vulnerability to affective disorders, genetic variability in serotonin (5-HT) and brain-derived neurotrophic factor (BDNF) expression has gained attention due to their association with anxiety-related functioning, but intermediate or endophenotypes remain to be identified. The serotonin transporter protein (5-HTT) regulates the amount and duration of 5-HT transmission by influencing reuptake. A polymorphism in the promoter region of the *SLC6A4* gene coding for 5-HTT, deemed 5-HTTLPR, results in two primary allelic variants, the long (L) and short (S) alleles, with the S alleles having lower 5-HTT transcription, resulting in reduced functional 5-HT reuptake and therefore increased 5-HT signaling (Lesch et al., 1996). Further, the L allele contains an A to G single-nucleotide polymorphism (SNP, rs25531) that influences transcriptional efficiency, rendering the L_G allele functionally similar to the S allele (Hu et al., 2006; Kenna et al., 2012). Behaviorally, relative to homozygous L carriers (LL), some studies have found that S carriers (SS or SL) have a higher prevalence of affective disorders, particularly in the setting of stressful life events (Caspi et al., 2003), depressive and anxiety-related traits (Gonda et al., 2009; Lesch et al., 1996; Telch et al., 2015), and stronger attentional bias towards emotional content (Beevers et al., 2009; Koizumi et al., 2010), however these findings are not always consistent. Higher emotional reactivity in S carriers is also supported by functional imaging studies of the amygdala, a region central to emotional processing (Phelps and LeDoux, 2005; Seeley et al., 2007), with greater amygdala activation (Caspi and Moffitt, 2006; Drabant et al., 2012; Hariri et al., 2002; Munafò et al., 2008) and atypical functional connectivity with ventromedial prefrontal, anterior cingulate, and insular cortices during emotional tasks or at rest (Heinz et al., 2005; Pezawas et al., 2005; Zhang et al., 2015). However, a recent meta-analysis has noted that the relationship between the S allele and amygdala activation may not be as

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robust as previously thought (Bastiaansen et al., 2014).

BDNF is a prototypical activity-dependent signaling molecule that has been linked to fear and anxiety regulation (Chen et al., 2006; Greenberg et al., 2009; Hill and Martinowich, 2016; Jiang et al., 2005; Montag et al., 2010; Ren-Patterson et al., 2005; Yu et al., 2012). It is controlled by the *BDNF* gene (Greenberg et al., 2009), which has a single-nucleotide polymorphism (SNP) substituting methionine (Met) for valine (Val). The Met allele reduces the release of BDNF from neurons and has been associated with higher prevalence of affective disorders (Jiang et al., 2005), anxiety-related traits (Chen et al., 2006; Montag et al., 2010; Ren-Patterson et al., 2005; Yu et al., 2012) and introversion (Terracciano et al., 2010), greater attentional bias towards emotional words (Gong et al., 2013), and increased amygdala (Gasic et al., 2009; Lau et al., 2010; Montag et al., 2008) and anterior cingulate activation during emotion processing (Martinowich and Lu, 2008). However, ValVal homozygosity has also been associated with higher prevalence of childhood-onset mood disorder (Strauss et al., 2005) and anxiety-related traits (Frustaci et al., 2008; Lang et al., 2005). Thus, both *BDNF Val66Met* alleles have been implicated as conferring vulnerability to affective disorders.

In addition to independent effects, 5-HTTLPR and *BDNF Val66Met* genotypes interactively influence vulnerability to psychopathology (Homberg et al., 2014; Martinowich and Lu, 2008; Pezawas et al., 2008), perhaps via effects on synaptic plasticity. The presence of the Met allele has been shown in studies to interact with both 5-HTTLPR alleles. Carriers of both the S and Met alleles have higher prevalence of stress and mood disorders (Bredemeier et al., 2014; Kaufman et al., 2006; Kim et al., 2007), increased anxiety symptoms (Kourmouli et al., 2013), and higher amygdala activation during emotion processing (Outhred et al., 2012). On the other hand, individuals carrying both the LL genotype and Met allele have higher neuroticism scores relative to non-Met carriers (Terracciano et al., 2010). Thus, the *BDNF Val66Met* polymorphism appears to moderate effects of 5-HTTLPR. BDNF secretion and transcription is controlled by neural activity and it is crucial for early serotonergic axonal growth and neuron development. Modifying its expression affects serotonin receptor binding in brain regions important for cognition and emotion (Djalali et al., 2005; Klein et al., 2010; Mamounas et al., 1995; Trajkovska et al., 2009). Conversely, serotonergic activity is thought to regulate BDNF expression through a feedback loop with serotonin receptors, as illustrated by findings of low BDNF expression in subjects with atypical serotonin transmission (Buchmann et al., 2013; Calabrese et al., 2013; Mattson et al., 2004; Molteni et al., 2010) and enhanced BDNF expression in response to selective serotonin reuptake inhibitors (De Foubert et al., 2004; Molteni et al., 2010). Genetic variability in these processes may mediate differences in emotional reactivity, which, in turn, may modulate synaptic plasticity of neural circuitry involved in emotional function.

In light of their interactive role in synaptic plasticity and emotion processing, we hypothesized that the interactive effects of 5-HTTLPR and *BDNF Val66Met* on anxiety would be mediated by cortical regions that are functionally connected to the amygdala, a structure of central importance to emotional function. This hypothesis is guided by the observation that coupling of functional activity across distant regions is shaped by synaptic plasticity (Hill and Martinowich, 2016). Since 5-HTTLPR and *BDNF Val66Met* interactively influence processes of synaptic plasticity, they ought to affect the strength of functional coupling in neural activity. Functional magnetic resonance imaging (fMRI) during a task-free resting state allows delineation of regions exhibiting functional coupling using temporal correlation in spontaneous neural activity – termed intrinsic functional connectivity (FC). Intrinsic functional networks of the brain recapitulate functional organization that is shaped by both genetic and experience-dependent processes (Raichle, 2010; Smith et al., 2009). The strength and extent of intrinsic amygdala FC is reflective of individual differences in symptoms of affective disorders as well as anxiety-related and personality factors in healthy subjects (Vaidya and Gordon, 2013). Therefore, if genetic variation influences emotional functioning and plasticity of underlying neural circuitry, those differences ought to be apparent in intrinsic amygdala FC networks. Such evidence will contribute towards identification of endophenotypes for emotional functioning, an important step towards describing causal pathways for vulnerability to affective disorders.

Resting state fMRI was performed in healthy adults who were genotyped for 5-HTTLPR and *BDNF Val66Met* polymorphisms and completed state and trait measures of anxiety. The anxiety assessment was not performed at the same time as the fMRI scan in order to avoid the acute effects of a stressful event, the impending MRI scan, from influencing the state anxiety assessment. Our goal was to examine the association between resting state amygdala connectivity strength and anxiety in a baseline state. We expected to replicate past findings of higher anxiety in S and Met carriers relative to other allelic combinations. We examined whether this genetic association with anxiety was mediated by regions that were functionally connected to the amygdala and sensitive to individual differences in anxiety.

2. Methods

2.1. Subjects

Ninety 18–23 year old students participated for payment. Each subject provided a saliva sample, completed the State-Trait Anxiety Inventory (STAI) Form Y (Spielberger, 1983), and weeks later underwent resting state fMRI. Exclusion criteria included self-reported (1) current or past psychiatric or neurological diagnoses; (2) use of psychotropic medication; (3) contraindications for MRI. Subjects filled a form that requested past or current psychiatric or neurological diagnoses and all current medications. Contraindications for MRI were queried with a standard institutional safety screening form. Subjects gave informed consent under the guidelines of the university Institutional Review Board. Ten subjects were excluded from data analysis due to extensive head motion ($n = 7$) during fMRI or incomplete STAI form ($n = 3$), yielding a final sample size of 80, with a mean age ± standard deviation of 19.9 ± 1.08 years and was 73.8% Non-Hispanic White, 15.0% Asian, 8.8% Hispanic, and 2.5% Non-Hispanic Black by ethnicity group. Subjects were designated to 4 groups based on 5-HTTLPR and

Table 1
Sample size, age, gender, and ethnicity distribution of the four groups by the 5-HTTLPR and *BDNF* genotype combinations. The groups did not differ by age ($F = 0.76$, $p = 0.52$), gender (Chi-square = 0.66, $p = 0.88$), or ethnicity (Chi-square = 11.4, $p = 0.25$). Distribution for 5-HTTLPR (SS: $n = 14$; SL: $n = 37$; LL: $n = 29$) and *BDNF Val66Met* (MetMet: $n = 7$; MetVal: $n = 29$; ValVal: $n = 44$) genotypes did not differ from Hardy–Weinberg equilibrium ($p = 0.93$ and $p = 0.79$, respectively).

	N	Age (Mean ± SD)	Males (%)	NH White (%)	NH Black (%)	Hispanic (%)	Asian (%)
SS/SL + MetMet/MetVal	25	19.77 ± 1.18	7 (28)	16 (64)	0 (0)	1 (4)	8 (32)
SS/SL + ValVal	26	19.96 ± 1.05	10 (38)	19 (73)	1 (4)	3 (12)	3 (12)
LL + MetMet/MetVal	11	19.63 ± 1.01	4 (36)	9 (82)	0 (0)	1 (9)	1 (9)
LL + ValVal	18	20.18 ± 1.07	6 (33)	15 (83)	1 (6)	2 (11)	0 (0)
Total	80	19.91 ± 1.09	27 (34)	59 (74)	2 (3)	7 (9)	12 (15)

NH = Non-Hispanic.

BDNF Val66Met genotype for the main analysis (refer to Table 1). The few subjects with L_AL_G ($N = 5$; no L_GL_G) were included in the LL group, which was consistent with the known low prevalence of the polymorphism in Caucasian populations (Hu et al., 2006). Genotype groups did not differ by gender ($p = 0.88$) and ethnicity ($p = 0.25$). Nevertheless, these variables were controlled for in all analyses.

2.2. Genotyping

Oragene saliva kits (DNA Genotek Inc., Ottawa, Ontario, Canada) were utilized to extract DNA from the saliva samples. The 5-HTTLPR polymorphism of the *SLC6A4* gene was genotyped by PCR using the DNA primers of concentration 10 μM: Forward: 5'-GGCCGTTGCCGCT CTGAATGC-3'; reverse: 5'-GAGGGACTGAGCTGGACAACCAC-3', generating 484- and 528-bp fragments corresponding to the short (S) and long (L) alleles. Further, the L_A and L_G alleles at the rs25531 SNP were identified by digesting the PCR products with a restriction enzyme MspI (New England BioLabs): bands at 340 bp for a L_AL_A homozygote, at 166 and 174 bp for a L_GL_G homozygote, and in between for a heterozygote. The Val66Met SNP (rs6265 variant) in the *BDNF* gene was processed using a Taqman Asay-on-Demand allele discrimination assay ID: C_11592758_10 (Life Technologies). PCR was completed using the AccuprimeTM Taq DNA polymerase system (Invitrogen) with the following PCR protocol: denaturation at 95 °C (10 min), then 35 cycles of denaturation at 95 °C (30 s), annealing at 65 °C (30 s), and extension at 72 °C (1 min), and followed by a final extension at 72 °C (10 min). Products were then run through electrophoresis on a 2% agarose gel stained with ethidium bromide and examined for evidence of the polymorphisms. For the 5-HTTLPR variant, a heterozygote sample with the S or L allele was identified. This sample was amplified with each set of samples and analyzed on the corresponding gel. For the *BDNF Val66Met* variant, we used genotype data available from the HapMap samples (CEU population, corresponding to a sample derived from Northern and Western European ancestry) to validate the genetic calls from the Taqman assay. The samples were purchased from Coriell (Camden, New Jersey). To ensure quality control, for each Taqman run on a 7900HT Real-Time PCR system, we also ran a wildtype and heterozygote sample along with the samples of interest.

2.3. Brain imaging

2.3.1. Acquisition

The resting state scan, lasting 5:04 min, was acquired by fMRI on a 3T Siemens Trio, as was conducted in the first in a series of scans which included task-evoked sessions (data published in Gordon et al., 2012). Using a gradient echo pulse sequence, 152 whole-brain images were acquired comprising 37 slices with parameters: TR/TE = 2000 ms/30 ms, FOV = 192 × 192 mm^2, 90° flip angle, 3 mm isotropic voxels. The first 4 images were discarded to allow for signal stabilization. Additionally, a high resolution T1-weighted structural scan (MPRAGE) was acquired with parameters: TR/TE = 2300 ms/2.94 ms, TI = 900 ms, 90° flip angle, 1 slab, 160 sagittal slices with a 1.0 mm thickness, FOV = 256 × 256 mm^2, matrix = 256 × 256, resulting in an effective resolution of 1.03 mm isotropic voxels.

2.3.2. Preprocessing

CONN functional connectivity toolbox (version 17.a, (Whitfield-Gabrieli and Nieto-Castanon, 2012) with SPM12 was used to perform standard preprocessing and de-noising steps, including slice-timing and motion correction, spatial normalization, smoothing (6 mm kernel), nuisance regression (6 motion parameters, first 5 principal components of CSF and white matter signal, and high motion volumes, i.e., greater than standard deviations of average signal or >0.5 mm framewise displacement (FD) "scrubbed" following Power et al. and bandpass filtering (0.008–0.09 Hz)(Power et al., 2012). All included subjects showed <2.0 mm of translational motion in any single direction (max = 0.78 mm) and <1.0° of rotation (max rotation = 0.85°) around any single axis. The percent of timepoints removed was very low on average (3.43 ± 6.70%) and did not differ by genotype (5-HTTLPR: $p = 0.91$, *BDNF:* $p = 0.61$). Further, percentage of timepoints removed and amount of FD did not correlate with anxiety (p's > 0.2).

2.3.3. Functional connectivity (FC) calculation

All FC analyses were conducted within the CONN toolbox. Seed regions of interest (ROIs) of the bilateral amygdala were created following the Harvard-Oxford Probability Atlas available for FSL in SPM12 thresholded at a probability of 0.75 (Left amygdala: 3368 mm^3, right amygdala: 3944 mm^3) (Desikan et al., 2006). For each seed region, mean time series was extracted across the voxels of the bilateral amygdala regions and correlated with that of every other voxel in the brain. Resulting connectivity measures were converted from r values to Z-scores in order to normalize the distribution of FC correlations across subjects. Thus, a subject-specific FC map was generated for each amygdala region and used in second level group analyses.

2.4. Behavioral analysis

2.4.1. Genotype effects on anxiety

Trait and state anxiety scaled scores tap dispositional and autonomic aspects of emotional functioning, respectively, and as expected, were highly correlated ($r = 0.65$, $p < 0.00001$). They were averaged (STAI-average) to generate one composite anxiety score per subject reflecting a comprehensive evaluation of anxiety as has been used in previous studies (Calabrese et al., 2008; Beaver et al., 2009; Haller et al., 2012). These scores were entered as the dependent measure into a 2 × 2 Analyses of Covariance (ANCOVA) with 5-HTTLPR (S carriers vs. LL) and *BDNF* (Met carriers vs. ValVal) as between-subjects factors, with gender and ethnicity as covariates. The same analysis was also performed on state and trait scores separately for the purpose of testing moderated mediation.

2.5. Imaging analysis

2.5.1. Anxiety effects on FC

Each subject's FC maps from both the right and left amygdala seeds were entered into a general linear regression model with STAI-average as the dependent measure, controlling for mean FD as well as gender and ethnicity. Specifically, it performs an F-test using an 'eye(2)' contrast which tests an OR conjunction of the left [1 0] and right [0 1] amygdala contrasts, to identify areas where the connectivity with any of the seed regions (left or right) is correlated with anxiety. Regions showing main effects of STAI-average were identified following correction for voxel-wise multiple comparisons at $p < 0.05$ using False Discovery Rate (FDR) correction for cluster level, which established the correction threshold at $p < 0.001$ and $k = 62$.

2.5.2. Moderated mediation analysis of anxiety-modulated functional connectivity

Significant regions from the above analysis (regions showing significant correlation with anxiety) were tested for moderated mediation of the 5-HTTLPR × *BDNF Val66Met* interaction with anxiety following 3 model tests (see schematic in Fig. 1). A regression analysis (Model 1) was first performed to examine the moderating effect of *BDNF Val66Met* on the association between 5-HTTLPR and anxiety scores. This analysis replicates the ANCOVA behavioral analysis described above but is necessary to test here in order to establish a direct effect of genetic association on anxiety in the context of mediation. Then, a base mediation model regression (Model 2) was performed to assess whether the regions identified above, a main effect of anxiety on FC, mediated the association between 5-HTTLPR and anxiety scores. FC from the left and right amygdala was tested separately in this step and only the model with significant mediation was considered further. This was followed

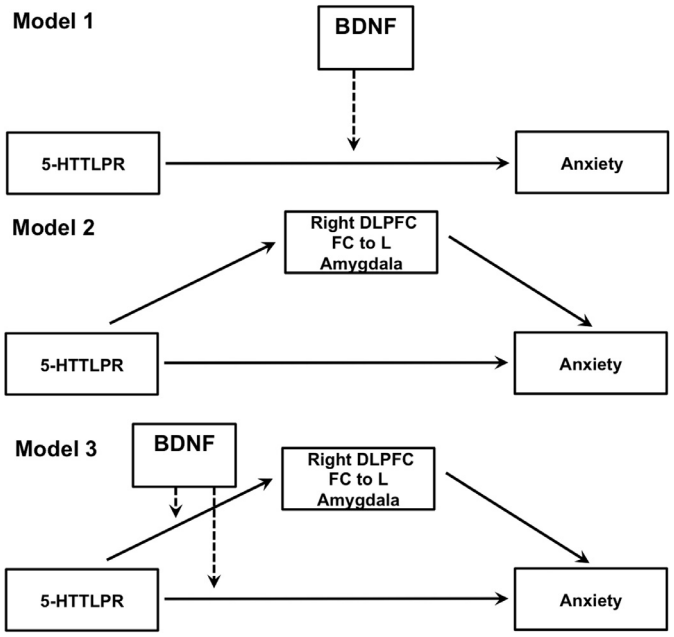

Fig. 1. Schema of the models used to test for amygdala-based functional connectivity (FC) moderated mediation of the 5-HTTLPR × *BDNF Val66Met* genotype interaction with anxiety. Model 1 tests the moderating effect of *BDNF Val66Met* on the association between 5-HTTLPR and anxiety. Model 2 tests the mediation effect of amygdala-based FC on the 5-HTTLPR genotype-anxiety association. Model 3 tests the moderation of the *BDNF Val66Met* genotype on the mediation effect of amygdala-based FC on the 5-HTTLPR genotype-anxiety association (Model 2). Gender and ethnicity were controlled in all 3 models.

by the moderated mediation analysis (Model 3) to test whether the mediation of FC of the association between 5-HTTLPR and anxiety was moderated by the *BDNF Val66Met* genotype. Average causal mediation effect (ACME) and 95% confidence intervals (CI) were reported for moderated mediation analyses. Gender and ethnicity were controlled in all 3 models. The causal mediation analysis with a nonparametric bootstrap method was conducted using the R statistical software (version 2.15, Vienna, Austria) mediation package (Tingley et al., 2014).

3. Results

3.1. Behavior

3.1.1. Genotype effects on anxiety

Overall, subjects had a mean STAI-average score of 46.74 (SD = 7.65, range = 30–69). A significant main effect of 5-HTTLPR was found on STAI-average such that scores (mean ± SD) were higher in S (47.87 ± 7.59) than LL (44.74 ± 7.48) carriers ($F(1, 74) = 4.49$, $p = 0.037$, partial eta-squared = 0.057), controlling for gender and ethnicity. While the main effect of *BDNF Val66Met* was not significant (Met: 46.76 ± 7.82; ValVal: 46.72 ± 7.60; $p = 0.48$), the 5-HTTLPR × *BDNF Val66Met* interaction was significant ($F(1, 74) = 4.22$, $p = 0.043$, partial eta-squared = 0.054), indicating that differences between S and LL carriers depended upon *BDNF Val66Met* alleles (see Fig. 2). Specifically, Met carriers with a S allele had higher anxiety than those with a LL allele ($t(34) = 2.83$, $p = 0.008$), whereas ValVal carriers did not differ by 5-HTTLPR ($t(42) = 0.08$, $p = 0.94$). Further, among S carriers, those with a Met allele did not have higher anxiety scores relative to their ValVal peers ($t(49) = 1.04$, $p = 0.30$), and LL carriers also did not differ by *BDNF Val66Met* ($t(27) = 1.79$, $p = 0.09$). Thus, the presence of the *BDNF* Met allele enhanced the difference in anxiety between S carriers and LL homozygotes of the 5-HTTLPR genotype.

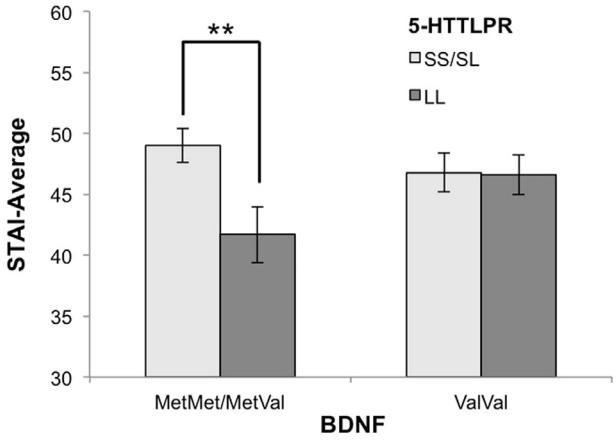

Fig. 2. STAI-average scores (mean ± SEM) were higher for short carriers (SS/SL) relative to homozygous long (LL) carriers of the 5-HTTLPR genotype in the presence of a *BDNF* Met allele. Scores did not differ between 5-HTTLPR allele groups for *BDNF* ValVal homozygotes. $^{**}p < 0.01$.

3.2. Brain imaging

3.2.1. Anxiety effects on FC

Whole-brain correlation of bilateral amygdala FC with anxiety scores at the corrected threshold revealed a significant region in the right dorsolateral prefrontal cortex (DLPFC, BA 9 with peak at $x = 40$, $y = 20$, $z = 32$; $k = 62$, peak $F(2150) = 14.45$, partial eta-squared = 0.16) such that higher FC was associated with higher anxiety scores (see Fig. 3). These results were not an artifact of our preprocessing pipeline as repeating the analysis with a global signal regression yielded the same right DLPFC region ($x = 38$, $y = 22$, $z = 32$, $k = 80$, peak $F(2150) = 15.38$, partial eta-squared = 0.17) for the main effect on anxiety at the $p < 0.05$ FDR-corrected threshold (see Fig. S1 in Supplementary Material). No other regions showed greater FC for higher anxiety with either the left or right amygdala at the corrected threshold.

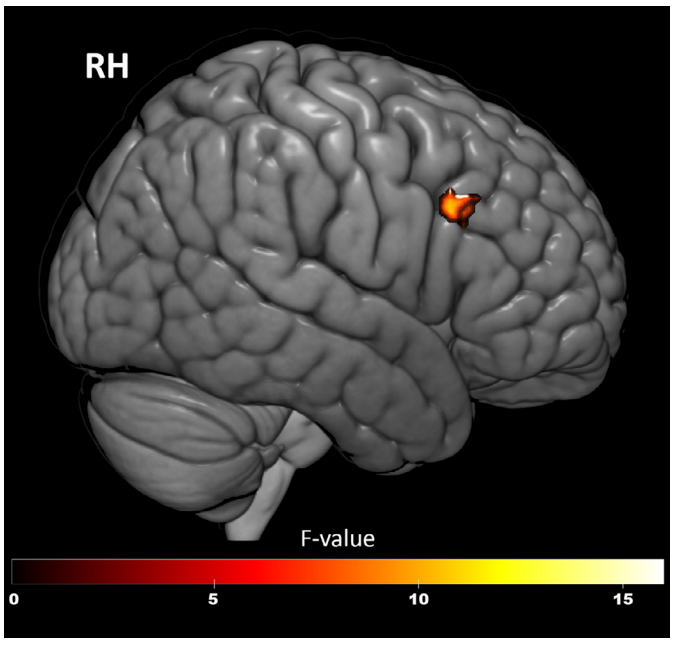

Fig. 3. Correlation of bilateral amygdala-seeded functional connectivity with anxiety scores. The significant cluster is located in the right dorsolateral prefrontal cortex (MNI coordinates: $x = 40$, $y = 20$, $z = 32$). Scale represents *F* statistic.

3.2.2. Moderated mediation analysis of anxiety-modulated functional connectivity

Through nonparametric bootstrap analysis for moderated mediation, we found that the FC of the left amygdala to right DLPFC demonstrated a significant mediating effect between the 5-HTTLPR gene and anxiety that was moderated by *BDNF Val66Met* genotype (see Fig. 1). Specifically, the first regression analysis (Model 1) replicated the association between 5-HTTLPR × *BDNF Val66Met* interaction and STAI-average scores. Both the main effect of 5-HTTLPR on anxiety (S carriers > LL homozygotes, $\beta = 0.99$, SE = 0.35, $p = 0.006$) and the 5-HTLLPR x *BDNF Val66Met* genotype interaction ($\beta = -1.0$, SE = 0.46, $p = 0.03$) were significant such that anxiety scores were higher in S allele carriers compared to LL homozygotes in the presence of the *BDNF* Met allele. The main effect of *BDNF Val66Met* on anxiety was not significant ($\beta = 0.72$, SE = 0.38, $p = 0.06$). Model 2 revealed a significant causal (indirect) mediation effect of left amygdala-right DLPFC FC on the association between 5-HTTLPR and anxiety (ACME = −2.15, 95% CI = −4.64–−0.19, $p = 0.04$) such that FC was higher in S carriers than LL homozygotes ($\beta = 0.57$, SE = 0.22, $p = 0.013$) and higher FC predicted higher anxiety ($\beta = 0.51$, SE = 0.10, $p < 0.0001$). The association between 5-HTTLPR genotype and anxiety became nonsignificant ($\beta = 0.12$, SE = 0.21, $p = 0.56$) when the left amygdala-right DLPFC FC term was added to in the model, indicating a full mediation. Model 2 for right amygdala-right DLPFC FC was not significant (ACME = −0.48, 95% CI = −1.72–0.82, $p = 0.36$) and thus was not considered further. Finally, Model 3 revealed a significant moderating effect of *BDNF Val66Met* genotype on the mediation of the effect of 5-HTTLPR on anxiety by left amygdala-right DLPFC FC (Model 2 above). The 5-HTTLPR genotype to FC relationship was still significant when accounting for *BDNF Val66Met* genotype such that S carriers still had higher FC than LL homozygotes ($\beta = 0.91$, SE = 0.35, $p = 0.012$). Further, the significant mediation observed in Model 2 was only present for Met carriers (ACME = −5.10, 95% CI = −11.2–−1.8, $p < 0.001$), but not for ValVal homozygotes (ACME = −1.28, 95% CI = −3.8–1.05, $p = 0.32$). The lack of mediation when *BDNF Val66Met* genotype was only ValVal homozygotes is likely due to the fact that S carriers did not differ in anxiety within the *BDNF* ValVal homozygotes.

Specifically, in LL homozygotes with lower reported anxiety than S carriers, FC correlated positively with anxiety regardless of their *BDNF Val66Met* allele status ($r = 0.72$ and 0.64, $p = 0.029$ and 0.007 for within Met carriers or Val homozygotes, respectively), controlling for gender and ethnicity (see Fig. 4). In contrast, the significant positive relationship between FC and anxiety was observed only in those with the Met allele for S carriers ($r = 0.43$, $p = 0.041$); FC and anxiety were uncorrelated in ValVal homozygotes ($r = 0.36$, $p = 0.084$).

Moderated mediation was additionally tested for state and trait anxiety separately. While state scores were normally distributed (Shapiro–Wilk $p = 0.12$), trait scores were not ($p = 0.009$) and, therefore, a log transformation was performed on trait scores, which resulted in a normal distribution ($W = 0.98$, $p = 0.30$). Separate testing of moderated mediation for state and log-transformed trait scores revealed similar results as those from the composite score, with some differences. Specifically, the direct effect (5-HTTLPR X BDNF interaction in anxiety) was significant for state scores ($p = 0.01$) and at trend level for trait scores ($p = 0.13$). The left amygdala-right DLPFC FC mediated fully the effect of 5-HTTLPR on state anxiety whereas it mediated partially the effect of 5-HTTLPR on trait anxiety. The moderating effect of *BDNF Val66Met* genotype on that mediation was significant for the trait measure and at trend level for the state measure. These results are detailed below.

The correlation of left amygdala-right DLPFC FC and state anxiety was also significant ($r = 0.40$, $p = 0.0002$). The FC of the left amygdala to right DLPFC demonstrated a significant mediating effect between the 5-HTTLPR gene and state anxiety that was moderated by *BDNF Val66Met* genotype (see schema in Fig. 1). Specifically, the first regression analysis (Model 1) revealed the association between 5-HTTLPR × *BDNF Val66Met* interaction and state anxiety scores. Both the main effect of 5-HTTLPR on state anxiety (S carriers > LL homozygotes, $\beta = 0.50$, $p = 0.03$) and the 5-HTLLPR x *BDNF Val66Met* genotype interaction ($\beta = -1.11$, $p = 0.01$) were significant such that anxiety scores were higher in S allele carriers compared to LL homozygotes in the presence of the *BDNF* Met allele. The main effect of *BDNF Val66Met* on state anxiety was not significant ($\beta = 0.66$, $p = 0.08$). Model 2 revealed a significant causal (indirect) mediation effect of left amygdala-right DLPFC FC on the association between 5-HTTLPR and state anxiety (ACME = −1.97 95% CI = −3.65–−0.44, $p < 0.001$) such that FC was higher in S carriers than LL homozygotes ($\beta = 0.57$, SE = 0.22, $p = 0.01$) and higher FC predicted higher anxiety ($\beta = 0.47$, SE = 0.10, $p < 0.0001$). The association between 5-HTTLPR genotype and state anxiety became nonsignificant ($\beta = 0.26$, SE = 0.22, $p = 0.22$) when the left amygdala-right DLPFC FC term was added to in the model, indicating a full mediation. Finally, Model 3 revealed a trend

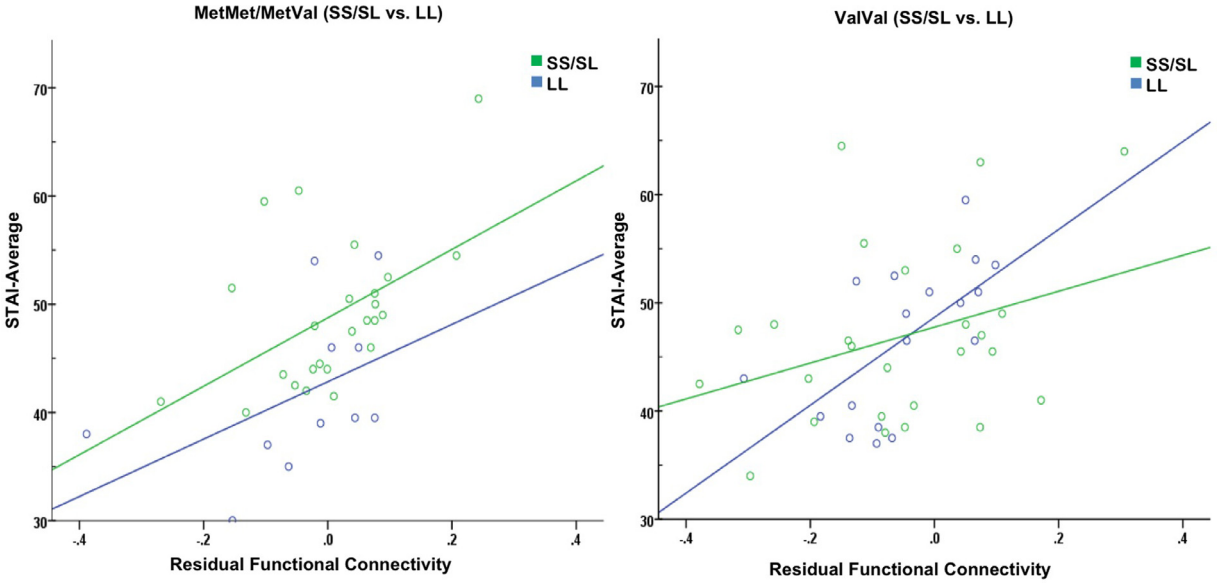

Fig. 4. Scatterplots of left amygdala-right dorsolateral prefrontal cortex (DLPFC) functional connectivity (FC) depicting the correlation of residual FC and anxiety scores (STAI-average) by 5-HTTLPR genotype within MetMet/MetVal *BDNF* genotype carriers (*left*) and within ValVal *BDNF* homozygotes (*right*).

level moderating effect of *BDNF Val66Met* genotype on the mediation of the effect of 5-HTTLPR on anxiety by left amygdala-right DLPFC FC (Model 2 in schema in Fig. 1). The 5-HTTLPR genotype to FC relationship was still significant when accounting for *BDNF Val66Met* genotype such that S carriers still had higher FC than LL homozygotes ($\beta = 0.91$, SE = 0.35, $p = 0.012$). Further, the trend level mediation observed in Model 2 was only present for Met carriers (ACME = −2.4, 95% CI = −6.7–0.57, $p = 0.12$), but not for ValVal homozygotes (ACME = −1.26, 95% CI = −4.04–1.25, $p = 0.29$).

The moderated mediation analysis for trait anxiety was performed on log-transformed scores. The correlation of left amygdala-right DLPFC FC and trait anxiety was also significant ($r = 0.48$, $p < 0.0001$). There was no direct effect of the 5-HTTLPR gene on trait anxiety (S carriers > LL homozygotes, $\beta = 0.25$, $p = 0.30$) and only trend level of moderating effect of *BDNF Val66Met* ($\beta = 0.84$, $p = 0.08$). However, Model 2, revealed a significant causal (indirect) mediation effect of left amygdala-right DLPFC FC on the association between 5-HTTLPR and anxiety (ACME = −0.04839, 95% CI = −0.10077–−0.01, $p = 0.008$) such that FC was higher in S carriers than LL homozygotes ($\beta = 0.57$, SE = 0.23, $p = 0.01$) and higher FC predicted higher anxiety ($\beta = 0.48$, SE = 0.10, $p < 0.0001$). The association between 5-HTTLPR genotype and anxiety became even more nonsignificant ($\beta = 0.03$, SE = 0.22, $p = 0.89$) when the left amygdala-right DLPFC FC term was added to the model, indicating a partial mediation. Finally, Model 3 revealed a significant moderating effect of *BDNF Val66Met* genotype on the mediation of the effect of 5-HTTLPR on anxiety by left amygdala-right DLPFC FC (Model 2 above). The 5-HTTLPR genotype to FC relationship was still significant when accounting for *BDNF Val66Met* genotype such that S carriers still had higher FC than LL homozygotes ($\beta = 0.91$, SE = 0.35 $p = 0.011$). Further, the significant mediation observed in Model 2 was only present for Met carriers (ACME = −0.1, 95% CI = −0.26–−0.01, $p = 0.032$), but not for ValVal homozygotes (ACME = −0.0236, 95% CI = −0.09–0.02, $p = 0.32$). The lack of mediation when *BDNF Val66Met* genotype was only ValVal homozygotes is likely due to the fact that S carriers did not differ in anxiety within the *BDNF* ValVal homozygotes.

4. Discussion

Interaction of 5-HTTLPR and *BDNF Val66Met* genetic polymorphisms was associated with anxiety in healthy individuals and this association was mediated by the intrinsic FC of the left amygdala with the right DLPFC. As expected, STAI-average anxiety scores were higher in S than LL carriers and this difference was augmented in the presence of the *BDNF* Met allele. Thus, functional integrity of this particular amygdala-prefrontal connection could be a potential intermediate phenotype for anxiety.

Behaviorally, *BDNF Val66Met* genotype moderated effects of 5-HTTLPR on anxiety. We tested the effect of genotypes on a composite measure of anxiety because it captures distinct dimensions of anxiety: the trait score assesses how prone one is to experience anxiety (Beiling et al., 1998), whereas the state score assesses autonomic aspects of emotional arousal to negative and positive affect. The two dimensions are interdependent, as those with higher trait anxiety tend to experience higher state anxiety reactions more often and with higher intensity (Edelmann, 1992; Spielberger et al., 1984). In order to include both dimensions of anxiety in our dependent measure, we tested the effect of genetic interaction and mediation by brain functioning on a combined measure of anxiety as utilized in various past studies testing effects of interventions on clinical anxiety (e.g., Calabrese et al., 2008; Beaver et al., 2009; Haller et al., 2012). Consistent with past reports, anxiety scores were higher in S than LL carriers (Gonda et al., 2009; Lesch et al., 1996), and, while the main effect of *BDNF Val66Met* was not significant, the effect of 5-HTTLPR genotype depended upon *BDNF Val66Met* such that differences were augmented in the presence of the Met allele, with highest scores in S and Met carriers and lowest scores in LL and Met carriers (Kourmouli et al., 2013). Although our sample was primarily female (66%), these findings were not driven by gender differences in anxiety as gender distribution did not differ by genetic groups, and, furthermore, was controlled for in the analysis along with variation in ethnicity.

With respect to anxiety, however, our results shed new light on the role of DLPFC and the status of its connection with the left amygdala. The right and left amygdala are postulated to differ in their contribution to emotional processing, with the right underlying a rapid but shallow dynamic stimulus detection whereas the left supporting sustained assessment of the stimulus (Markowitsch, 1998). Support for these putative roles arises from meta-analysis of task differences in amygdala response, with those activating the right amygdala requiring rapid detection (e.g., masked stimuli) and those activating the left amygdala requiring sustained emotional processing (e.g., reading words) (Costafreda et al., 2008). DLPFC has been associated with anxiety in past studies, with anxious subjects having greater DLPFC activation in tasks requiring higher attentional control compared to non-anxious individuals (Basten et al., 2011; Gold et al., 2015; Sylvester et al., 2012). Further, Roy et al. found that a subdivision of the amygdala, the superficial amygdala, had positive intrinsic FC with the right DLPFC in subjects with generalized anxiety disorder (GAD), while controls had a negative FC relationship (Roy et al., 2013). Etkin et al. similarly reported increased connectivity between both the left and right amygdala and the right DLPFC for GAD patients relative to controls during a task-free resting state (Etkin et al., 2009). The right lateral PFC has been implicated in the down regulation of emotion as examined by studies utilizing cognitive reappraisal paradigms (Ochsner et al., 2004), suggesting an inhibitory role in emotional processing. Phenotypically, higher anxiety in GAD relative to healthy subjects, or anxiety at the higher end of a normal continuum as in the present sample, manifests as more effortful regulation of emotional reactivity. However, other studies have noted decreased intrinsic FC between the amygdala and other areas of emotional processing such as the ventromedial and orbitofrontal PFC in subjects with high anxiety (Kim et al., 2010; Hahn et al., 2011). In light of the present findings, it may be that stronger intrinsic FC between the right DLPFC and left amygdala enables this capacity for effortful regulation of emotion in those vulnerable to anxiety. Task-based studies are needed to evaluate whether this particular cross-hemispheric connection is also implicated during emotionally arousing experiences.

The left amygdala-right DLPFC connection was found to correlate with anxiety levels, and significantly mediated the interactive effects of 5-HTTLPR and *BDNF Val66Met* on anxiety. The scatterplots in Fig. 4 elucidate the influence of *BDNF Val66Met* on the correlation between anxiety scores and FC by 5-HTTLPR genotype. Testing for moderated mediation separately for trait and state measures revealed similar results as the composite measure, albeit with slight differences in significance. It is important to note that our sample was healthy, without clinically significant levels of anxiety. Therefore, conclusions about how these results may be informative for vulnerability to anxiety disorders cannot be drawn.

However, these results point to the mutual influence of serotonin and BDNF signaling on integrity of functional interaction between the amygdala and DLPFC as a source of significant variance in anxiety levels. Developmental neuronal plasticity is strongly influenced by interacting expression of serotonin and BDNF and, thus, the observed findings may reflect individual differences established during development (Buchmann et al., 2013; Calabrese et al., 2013; Djalali et al., 2005; Klein et al., 2010; Mamounas et al., 1995; Mattson et al., 2004; Molteni et al., 2010; Trajkovska et al., 2009). Conversely, plasticity of these and other structures is demonstrated by interaction of these genotypes with early life stress and their association with anxiety or depressive symptoms (Caspi et al., 2003; Gatt et al., 2009). While we did not measure any potential environmental factors, it is likely that they account for additional variance in the association between FC and

anxiety levels.

While a significant moderated mediation was observed for the left amygdala to right DLPFC, reasons why a similar finding was not found with the right amygdala are less clear. The indirect model (Model 2) for right amygdala-right DLPFC FC was not significant and thus was not tested for moderated mediation of the genotype-anxiety association. Reviews of specific functional roles of the left and right amygdala have noted that the left amygdala activation is more frequently implicated in emotional processing studies, but the evidence of lateralization in emotional regulation has been inconsistent (Baas et al., 2004; Chen et al., 2014; Markowitsch, 1998). The lack of contralateral finding with the right amygdala may stem from an asymmetric functional contribution from each amygdala in emotional regulation, which should be examined in future studies of amygdala-based neural circuitry on function. Also, of note, we did not observe genetic differences in intrinsic FC with the insula, which has been implicated in generalized anxiety disorder (GAD) (Baur et al., 2013; Dalton et al., 2014; Roy et al., 2013; Stein et al., 2007) and was found to mediate 5-HTTLPR effects on anxiety in healthy Han Chinese males (Zhang et al., 2015). Insensitivity of amygdala-insula FC to genetic differences in the present sample may relate to its composition, which consisted of mainly Caucasian females without a diagnosis of GAD.

The observed results must be interpreted in the context of the following factors: first, anxiety was measured at the time of saliva collection several weeks prior to MRI, in order to avoid inflated estimation induced by anxiety due to an imminent MRI scan. Second, head motion did not contribute to the observed FC differences as it did not differ by genotype and we employed currently recommended controls (Cox et al., 2017). Third, unlike some recent studies that have included 5-HTTLPR long allele adenine to guanine SNP (L_G) carriers with S carriers as they are deemed functionally similar (Hu et al., 2006; Kenna et al., 2012), we included L_AL_G carriers in the LL group because they were few in number ($N = 5$; no L_GL_G), consistent with reports of low prevalence in Caucasian populations (Hu et al., 2006). This inclusion, however, would only serve to reduce and not enhance S vs. LL differences. Lastly, while our sample size is larger than the majority of past fMRI studies of these genotypes (e.g., $N = 18$–92 (Lau et al., 2010; Montag et al., 2008; Munafò et al., 2008; Wei et al., 2017), it is important to note that cell sizes for examining effects of two genes are small. Small sample sizes for genetic studies pose the risk of false-positive results, and, therefore, our findings should be considered preliminary and replication with larger samples would be highly desirable to increase the power of the gene by environmental findings in these early analyses.

Our results in combination with past findings linking interaction of 5-HTTLPR and *BDNF Val66Met* genotypes with affective psychopathology suggest that functional connectivity between left amygdala with right DLPFC may serve as an intermediate phenotype for anxiety (Bredemeier et al., 2014; Kaufman et al., 2006; Kim et al., 2007; Kourmouli et al., 2013; Outhred et al., 2012). Studies replicating the present findings in individuals with anxiety disorders can more definitively establish its intermediate or endophenotype status.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.pscychresns.2019.01.010.

References

Baas, D., Aleman, A., Kahn, R.S., 2004. Lateralization of amygdala activation: a systematic review of functional neuroimaging studies. Brain Res. Rev. 45 (2), 96–103.
Basten, U., Stelzel, C., Fiebach, C.J., 2011. Trait anxiety modulates the neural efficiency of inhibitory control. J. Cogn. Neurosci. 23 (10), 3132–3145.
Bastiaansen, J.A., Servaas, M.N., Marsman, J.B.C., Ormel, J., Nolte, I.M., Riese, H., et al., 2014. Filling the gap: relationship between the serotonin-transporter-linked polymorphic region and amygdala activation. Psychol. Sci. 25 (11), 2058–2066.
Baur, V., Hänggi, J., Langer, N., Jäncke, L., 2013. Resting-state functional and structural connectivity within an insula–amygdala route specifically index state and trait anxiety. Biol. Psychiatry 73 (1), 85–92.
Beaver, K., Tysver-Robinson, D., Campbell, M., Twomey, M., Williamson, S., Hindley, A., et al., 2009. . Comparing hospital and telephone follow-up after treatment for breast cancer: randomised equivalence trial. BMJ 338, a3147.
Beevers, C.G., Wells, T.T., Ellis, A.J., McGeary, J.E., 2009. Association of the serotonin transporter gene promoter region (5-HTTLPR) polymorphism with biased attention for emotional stimuli. J. Abnorm. Psychol. 118 (3), 670.
Bredemeier, K., Beevers, C.G., McGeary, J.E., 2014. Serotonin transporter and BDNF polymorphisms interact to predict trait worry. Anxiety Stress Coping 27 (6), 712–721.
Beiling, P.J., Antony, M.M., Swinson, R.P., 1998. The state-trait anxiety inventory, trait version: structure and content re-examined. Behav. Res. Ther. 36, 777–788.
Buchmann, A.F., Hellweg, R., Rietschel, M., Treutlein, J., Witt, S.H., Zimmermann, U.S., et al., 2013. BDNF Val 66 Met and 5-HTTLPR genotype moderate the impact of early psychosocial adversity on plasma brain-derived neurotrophic factor and depressive symptoms: a prospective study. Eur. Neuropsychopharmacol. 23 (8), 902–909.
Calabrese, C., Gregory, W.L., Leo, M., Kraemer, D., Bone, K., Oken, B., 2008. Effects of a standardized *Bacopa monnieri* extract on cognitive performance, anxiety, and depression in the elderly: a randomized, double-blind, placebo-controlled trial. J. Altern. Complement. Med. 14 (6), 707–713.
Calabrese, F., Guidotti, G., Middelman, A., Racagni, G., Homberg, J., Riva, M.A., 2013. Lack of serotonin transporter alters BDNF expression in the rat brain during early postnatal development. Mol. Neurobiol. 48 (1), 244–256.
Caspi, A., Moffitt, T.E., 2006. Gene-environment interactions in psychiatry: joining forces with neuroscience. Nat. Rev. Neurosci. 7 (7), 583.
Caspi, A., Sugden, K., Moffitt, T.E., Taylor, A., Craig, I.W., Harrington, H., et al., 2003. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 301 (5631), 386–389.
Chen, Y.T., Huang, M.W., Hung, I.C., Lane, H.Y., Hou, C.J., 2014. Right and left amygdalae activation in patients with major depression receiving antidepressant treatment, as revealed by fMRI. Behav. Brain Funct. 10 (1), 36.
Chen, Z.Y., Jing, D., Bath, K.G., Ieraci, A., Khan, T., Siao, C.J., et al., 2006. Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. Science 314 (5796), 140–143.
Costafreda, S.G., Brammer, M.J., David, A.S., Fu, C.H., 2008. Predictors of amygdala activation during the processing of emotional stimuli: a meta-analysis of 385 PETand fMRI studies. Brain Res. Rev. 58 (1), 57–70.
Cox, R.W., Chen, G., Glen, D.R., Reynolds, R.C., Taylor, P.A., 2017. FMRI clustering in AFNI: false-positive rates redux. Brain Connect. 7 (3), 152–171.
Dalton, E.D., Hammen, C.L., Najman, J.M., Brennan, P.A., 2014. Genetic susceptibility to family environment: BDNF Val66met and 5-HTTLPR influence depressive symptoms. J. Fam. Psychol. 28 (6), 947.
De Foubert, G., Carney, S.L., Robinson, C.S., Destexhe, E.J., Tomlinson, R., Hicks, C.A., et al., 2004. Fluoxetine-induced change in rat brain expression of brain-derived neurotrophic factor varies depending on length of treatment. Neuroscience 128 (3), 597–604.
Desikan, R.S., Ségonne, F., Fischl, B., Quinn, B.T., Dickerson, B.C., Blacker, D., et al., 2006. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. Neuroimage 31 (3), 968–980.
Djalali, S., Höltje, M., Grosse, G., Rothe, T., Stroh, T., Grosse, J., et al., 2005. Effects of brain-derived neurotrophic factor (BDNF) on glial cells and serotonergic neurones during development. J. Neurochem. 92 (3), 616–627.
Drabant, E.M., Ramel, W., Edge, M.D., Hyde, L.W., Kuo, J.R., Goldin, P.R., et al., 2012. Neural mechanisms underlying 5-HTTLPR-related sensitivity to acute stress. Am. J. Psychiatr. 169 (4), 397–405.
Edelmann, R.J., 1992. Conceptual Issues. In: Edelmann, R.J. (Ed.), Anxiety: Theory, Research, and Intervention in Clinical and Healthy Psychology. Wiley, Chichester, pp. 1–18.
Etkin, A., Prater, K.E., Schatzberg, A.F., Menon, V., Greicius, M.D., 2009. Disrupted amygdalar subregion functional connectivity and evidence of a compensatory network in generalized anxiety disorder. Arch. Gen. Psychiat. 66 (12), 1361–1372.
Frustaci, A., Pozzi, G., Gianfagna, F., Manzoli, L., Boccia, S., 2008. Meta-analysis of the brain-derived neurotrophic factor gene (BDNF) Val66Met polymorphism in anxiety disorders and anxiety-related personality traits. Neuropsychobiology 58 (3–4), 163–170.
Gasic, G.P., Smoller, J.W., Perlis, R.H., Sun, M., Lee, S., Kim, B.W., et al., 2009. BDNF, relative preference, and reward circuitry responses to emotional communication. Am. J. Med. Genet. Part B: Neuropsychiatr. Genet. 150 (6), 762–781.
Gatt, J.M., Nemeroff, C.B., Dobson-Stone, C., Paul, R.H., Bryant, R.A., Schofield, P.R., et al., 2009. Interactions between BDNF Val66Met polymorphism and early life stress predict brain and arousal pathways to syndromal depression and anxiety. Mol. Psychiatry 14 (7), 681.
Gold, A.L., Morey, R.A., McCarthy, G., 2015. Amygdala-prefrontal cortex functional connectivity during threat-induced anxiety and goal distraction. Biol. Psychiatry 77

(4), 394–403.
Gonda, X., Fountoulakis, K.N., Juhasz, G., Rihmer, Z., Lazary, J., Laszik, A., et al., 2009. Association of the s allele of the 5-HTTLPR with neuroticism-related traits and temperaments in a psychiatrically healthy population. Eur. Arch. Psychiatry Clin. Neurosci. 259 (2), 106–113.
Gong, P., Xi, S., Li, S., Cao, G., Zhang, P., Shen, G., et al., 2013. Effect of Val66Met polymorphism in BDNF on attentional bias in an extroverted Chinese Han population. Acta Neurobiol. Exp. (Wars) 73, 280–288.
Gordon, E.M., Stollstorff, M., Devaney, J.M., Bean, S., Vaidya, C.J., 2012. Effect of dopamine transporter genotype on intrinsic functional connectivity depends on cognitive state. Cereb. Cortex 22 (9), 2182–2196.
Greenberg, M.E., Xu, B., Lu, B., Hempstead, B.L., 2009. New insights in the biology of BDNF synthesis and release: implications in CNS function. J. Neurosci. 29 (41), 12764–12767.
Hahn, A., Stein, P., Windischberger, C., Weissenbacher, A., Spindelegger, C., Moser, E., 2011. Reduced resting-state functional connectivity between amygdala and orbitofrontal cortex in social anxiety disorder. Neuroimage 56 (3), 881–889.
Haller, J., Freund, T.F., Gyimesi, K., Furedi, J., Krecsak, L., Zambori, J., 2012. The anxiolytic potentional and psychotropic side effects of an *Echinacea* preparation in laboratory animals and healthy volunteers. Phytother. Res. 27 (1), 54–61.
Hariri, A.R., Mattay, V.S., Tessitore, A., Kolachana, B., Fera, F., Goldman, D., et al., 2002. Serotonin transporter genetic variation and the response of the human amygdala. Science 297 (5580), 400–403.
Heinz, A., Braus, D.F., Smolka, M.N., Wrase, J., Puls, I., Hermann, D., et al., 2005. Amygdala-prefrontal coupling depends on a genetic variation of the serotonin transporter. Nat. Neurosci. 8 (1), 20.
Hill, J.L., Martinowich, K., 2016. Activity-dependent signaling: influence on plasticity in circuits controlling fear-related behavior. Curr. Opin. Neurobiol. 36, 59–65.
Homberg, J.R., Molteni, R., Calabrese, F., Riva, M.A., 2014. The serotonin–BDNF duo: developmental implications for the vulnerability to psychopathology. Neurosci. Biobehav. Rev. 43, 35–47.
Hu, X.Z., Lipsky, R.H., Zhu, G., Akhtar, L.A., Taubman, J., Greenberg, B.D., et al., 2006. Serotonin transporter promoter gain-of-function genotypes are linked to obsessive-compulsive disorder. Am. J. Hum. Genet. 78 (5), 815–826.
Jiang, X., Xu, K., Hoberman, J., Tian, F., Marko, A.J., Waheed, J.F., et al., 2005. BDNF variation and mood disorders: a novel functional promoter polymorphism and Val66Met are associated with anxiety but have opposing effects. Neuropsychopharmacology 30 (7), 1353.
Kaufman, J., Yang, B.Z., Douglas-Palumberi, H., Grasso, D., Lipschitz, D., Houshyar, S., et al., 2006. Brain-derived neurotrophic factor–5-HTTLPR gene interactions and environmental modifiers of depression in children. Biol. Psychiatry 59 (8), 673–680.
Kenna, G.A., Roder-Hanna, N., Leggio, L., Zywiak, W.H., Clifford, J., Edwards, S., et al., 2012. Association of the 5-HTT gene-linked promoter region (5-HTTLPR) polymorphism with psychiatric disorders: review of psychopathology and pharmacotherapy. Pharmgenomics Pers. Med. 5, 19.
Kim, J.M., Gee, D.G., Loucks, R.A., Davis, F.C., Whalen, P.J., 2010. Anxiety dissociates dorsal and ventral medial prefrontal cortex functional connectivity with the amygdala at rest. Cereb. Cortex 21 (7), 1667–1673.
Kim, J.M., Stewart, R., Kim, S.W., Yang, S.J., Shin, I.S., Kim, Y.H., et al., 2007. Interactions between life stressors and susceptibility genes (5-HTTLPR and BDNF) on depression in Korean elders. Biol. Psychiatry 62 (5), 423–428.
Klein, A.B., Santini, M.A., Aznar, S., Knudsen, G.M., Rios, M., 2010. Changes in 5-HT2A-mediated behavior and 5-HT2A-and 5-HT1A receptor binding and expression in conditional brain-derived neurotrophic factor knock-out mice. Neuroscience 169 (3), 1007–1016.
Koizumi, A., Kitagawa, N., Kitamura, M.S., Kondo, H.M., Sato, T., Kashino, M., 2010. Serotonin transporter gene and inhibition of conflicting emotional information. Neuroreport 21 (6), 422–426.
Kourmouli, N., Samakouri, M., Mamatsiou, A., Trypsianis, G., Livaditis, M., Veletza, S., 2013. Effect of BDNF Val66Met and serotonin transporter 5-HTTLPR polymorphisms on psychopathological characteristics in a sample of university students. Psychiatr. Genet. 23 (5), 188–197.
Lang, U.E., Hellweg, R., Kalus, P., Bajbouj, M., Lenzen, K.P., Sander, T., et al., 2005. Association of a functional BDNF polymorphism and anxiety-related personality traits. Psychopharmacology 180 (1), 95–99.
Lau, J.Y., Goldman, D., Buzas, B., Hodgkinson, C., Leibenluft, E., Nelson, E., et al., 2010. BDNF gene polymorphism (Val66Met) predicts amygdala and anterior hippocampus responses to emotional faces in anxious and depressed adolescents. Neuroimage 53 (3), 952–961.
Lesch, K.P., Bengel, D., Heils, A., Sabol, S.Z., Greenberg, B.D., Petri, S., et al., 1996. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 274 (5292), 1527–1531.
Mamounas, L.A., Blue, M.E., Siuciak, J.A., Altar, C.A., 1995. Brain-derived neurotrophic factor promotes the survival and sprouting of serotonergic axons in rat brain. J. Neurosci. 15 (12), 7929–7939.
Markowitsch, H.J., 1998. Differential contribution of right and left amygdala to affective information processing. Behav. Neurol. 11 (4), 233–244.
Martinowich, K., Lu, B., 2008. Interaction between BDNF and serotonin: role in mood disorders. Neuropsychopharmacology 33 (1), 73.
Mattson, M.P., Maudsley, S., Martin, B., 2004. BDNF and 5-HT: a dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. Trends Neurosci. 27 (10), 589–594.
Molteni, R., Cattaneo, A., Calabrese, F., Macchi, F., Olivier, J.D., Racagni, G., et al., 2010. . Reduced function of the serotonin transporter is associated with decreased expression of BDNF in rodents as well as in humans. Neurobiol. Dis. 37 (3), 747–755.
Montag, C., Basten, U., Stelzel, C., Fiebach, C.J., Reuter, M., 2010. The BDNF Val66Met polymorphism and anxiety: support for animal knock-in studies from a genetic association study in humans. Psychiat. Res. 179 (1), 86–90.
Montag, C., Reuter, M., Newport, B., Elger, C., Weber, B., 2008. The BDNF Val66Met polymorphism affects amygdala activity in response to emotional stimuli: evidence from a genetic imaging study. Neuroimage 42 (4), 1554–1559.
Munafò, M.R., Brown, S.M., Hariri, A.R., 2008. Serotonin transporter (5-HTTLPR) genotype and amygdala activation: a meta-analysis. Biol. Psychiatry 63 (9), 852–857.
Ochsner, K.N., Ray, R.D., Cooper, J.C., Roberston, E.R., Chopra, S., Gabrieli, J.D., Gross, J.J., 2004. For better or for worse: neural systems supporting the cognitive down- and up-regulation of negative emotion. Neuroimage 23 (2), 483–499.
Outhred, T., Das, P., Dobson-Stone, C., Griffiths, K., Felmingham, K.L., Bryant, R.A., et al., 2012. The functional epistasis of 5-HTTLPR and BDNF Val66Met on emotion processing: a preliminary study. Brain Behav. 2 (6), 778–788.
Pezawas, L., Meyer-Lindenberg, A., Drabant, E.M., Verchinski, B.A., Munoz, K.E., Kolachana, B.S., et al., 2005. 5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: a genetic susceptibility mechanism for depression. Nat. Neurosci. 8 (6), 828.
Pezawas, L., Meyer-Lindenberg, A., Goldman, A.L., Verchinski, B.A., Chen, G., Kolachana, B.S., et al., 2008. Evidence of biologic epistasis between BDNF and SLC6A4 and implications for depression. Mol. Psychiatry 13 (7), 709.
Phelps, E.A., LeDoux, J.E., 2005. Contributions of the amygdala to emotion processing: from animal models to human behavior. Neuron 48 (2), 175–187.
Power, J.D., Barnes, K.A., Snyder, A.Z., Schlaggar, B.L., Petersen, S.E., 2012. Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. Neuroimage 59 (3), 2142–2154.
Raichle, M.E., 2010. Two views of brain function. Trends Cogn. Sci. 14 (4), 180–190.
Ren-Patterson, R.F., Cochran, L.W., Holmes, A., Sherrill, S., Huang, S.J., Tolliver, T., et al., 2005. Loss of brain-derived neurotrophic factor gene allele exacerbates brain monoamine deficiencies and increases stress abnormalities of serotonin transporter knockout mice. J. Neurosci. Res. 79 (6), 756–771.
Roy, A.K., Fudge, J.L., Kelly, C., Perry, J.S., Daniele, T., Carlisi, C., et al., 2013. Intrinsic functional connectivity of amygdala-based networks in adolescent generalized anxiety disorder. J. Am. Acad. Child Adolesc. Psychiatry 52 (3), 290–299.
Seeley, W.W., Menon, V., Schatzberg, A.F., Keller, J.G., Over, G.H., Kenna, H., et al., 2007. Dissociable intrinsic connectivity networks for salience processing and executive control. J. Neurosci. 27 (9), 2349–2356.
Smith, S.M., Fox, P.T., Miller, K.L., Glahn, D.C., Fox, P.M., Mackay, C.E., et al., 2009. Correspondence of the brain's functional architecture during activation and rest. Proc. Natl. Acad. Sci. USA 106 (31), 13040–13045.
Spielberger, C.D., 1983. Manual for the State-Trait Anxiety Inventory STAI (form Y)("Self-evaluation Questionnaire").
Spielberger, C.D., Pollans, C.H., Worden, T.V., 1984. Adult psychopathology and diagnosis. In: Turner, S.M., Hersen, M. (Eds.), Anxiety Disorders. Wiley, New York, pp. 263–303.
Stein, M.B., Simmons, A.N., Feinstein, J.S., Paulus, M.P., 2007. Increased amygdala and insula activation during emotion processing in anxiety-prone subjects. Am. J. Psychiatr. 164 (2), 318–327.
Strauss, J., Barr, C.L., George, C.J., Devlin, B., Vetro, A., Kiss, E., et al., 2005. . Brain-derived neurotrophic factor variants are associated with childhood-onset mood disorder: confirmation in a Hungarian sample. Mol. Psychiatry 10 (9), 861.
Sylvester, C.M., Corbetta, M., Raichle, M.E., Rodebaugh, T.L., Schlaggar, B.L., Sheline, Y.I., et al., 2012. Functional network dysfunction in anxiety and anxiety disorders. Trends Neurosci. 35 (9), 527–535.
Telch, M.J., Beevers, C.G., Rosenfield, D., Lee, H.J., Reijntjes, A., Ferrell, R.E., et al., 2015. 5-HTTLPR genotype potentiates the effects of war zone stressors on the emergence of PTSD, depressive and anxiety symptoms in soldiers deployed to Iraq. World Psychiatry 14 (2), 198–206.
Terracciano, A., Tanaka, T., Sutin, A.R., Deiana, B., Balaci, L., Sanna, S., et al., 2010. BDNF Val66Met is associated with introversion and interacts with 5-HTTLPR to influence neuroticism. Neuropsychopharmacology 35 (5), 1083.
Tingley, D., Yamamoto, T., Hirose, K., Imai, K., Keele, L., 2014. Mediation: R package for causal mediation analysis. J. Stat. Softw. 59 (5), 1–38.
Trajkovska, V., Santini, M.A., Marcussen, A.B., Thomsen, M.S., Hansen, H.H., Mikkelsen, J.D., et al., 2009. BDNF downregulates 5-HT2A receptor protein levels in hippocampal cultures. Neurochem. Int. 55 (7), 697–702.
Vaidya, C.J., Gordon, E.M., 2013. Phenotypic variability in resting-state functional connectivity: current status. Brain Connect. 3 (2), 99–120.
Wei, S.M., Eisenberg, D.P., Nabel, K.G., Kohn, P.D., Kippenhan, J.S., Dickinson, D., et al., 2017. Brain-derived neurotrophic factor Val66Met polymorphism affects the relationship between an anxiety-related personality trait and resting regional cerebral blood flow. Cereb. Cortex 27 (3), 2175–2182.
Whitfield-Gabrieli, S., Nieto-Castanon, A., 2012. Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks. Brain Connect 2 (3), 125–141.
Yu, H., Wang, D.D., Wang, Y., Liu, T., Lee, F.S., Chen, Z.Y., 2012. Variant brain-derived neurotrophic factor Val66Met polymorphism alters vulnerability to stress and response to antidepressants. J. Neurosci. 32 (12), 4092–4101.
Zhang, L., Liu, L., Li, X., Song, Y., Liu, J., 2015. Serotonin transporter gene polymorphism (5-HTTLPR) influences trait anxiety by modulating the functional connectivity between the amygdala and insula in Han Chinese males. Hum. Brain Mapp. 36 (7), 2732–2742.