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Original Article

ORIGINAL ARTICLE

Effects of Serotonin Transporter Gene Variation on Impulsivity Mediated by Default Mode Network: A Family Study of Depression

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Abstract

Serotonergic neurotransmission, potentially through effects on the brain's default mode network (DMN), may regulate aspects of attention including impulse control. Indeed, genetic variants of the serotonin transporter (5-HTT) have been implicated in impulsivity and related psychopathology. Yet it remains unclear the mechanism by which the 5-HTT genetic variants contribute to individual variability in impulse control. Here, we tested whether DMN connectivity mediates an association between the 5-HTT genetic variants and impulsivity. Participants (N = 92) were from a family cohort study of depression in which we have previously shown a broad distribution of 5-HTT variants. We genotyped for 5-HTTLPR and rs25531 (stratified by transcriptional efficiency: 8 low/low, 53 low/high, and 31 high/high), estimated DMN structural connectivity using diffusion probabilistic tractography, and assessed behavioral measures of impulsivity (from 12 low/low, 48 low/high, and 31 high/high) using the Continuous Performance Task. We found that low transcriptional efficiency genotypes were associated with decreased connection strength between the posterior DMN and the superior frontal gyrus (SFG). Path modeling demonstrated that decreased DMN–SFG connectivity mediated the association between low-efficiency genotypes and increased impulsivity. Taken together, this study suggests a gene-brain-behavior pathway that perhaps underlies the role of the serotonergic neuromodulation in impulse control.

Key words: 5-HTTLPR, commission error, continuous performance task, diffusion probabilistic tractography, mediation

Introduction

The short (S) allele of the serotonin transporter (5-HTT) linked polymorphic region (5-HTTLPR) in SLC6A4 lowers the transcriptional efficiency of the gene encoding 5-HTT. Studies have associated low-efficiency 5-HTTLPR with depression (Caspi et al. 2003; Hariri and Holmes 2006; Karg et al. 2011) and other forms of psychopathology such as attention-deficit-hyperactivity disorder (ADHD) (Banerjee et al. 2006), alcohol dependence (van der Zwaluw et al. 2010), and schizophrenia (Malhotra et al. 1998). There are, however, conflicting reports with some studies showing no association with depression (Risch et al. 2009), and others suggesting associations with high, rather than low, efficiency genotypes (Bleich et al. 2007; Gorodetsky et al. 2016). These mixed reports are perhaps not surprising given the complexity of the clinical phenotype of depression with its effects on mood, cognitive, circadian, and endocrine systems (Hasler et al. 2004; Hasler and Northoff 2011), and suggest that depression's underlying genetic profile may be equally complex.

Focusing on endophenotypes may be a more fruitful strategy to examining genetic associations. Endophenotypes are trait markers that are objectively quantifiable and, at least in theory, are more proximal in their biological pathway to the underlying genetics than is the clinical phenotype (Gottesman and Gould 2003). Here, we leverage a family-based cohort study of individuals at high and low familial risk for depression to examine associations across genetic, neural, and behavioral data with the goal of identifying a potential endophenotype of depression at the level of brain and behavior.

Human neuroimaging studies have suggested serotonin as an important neuromodulator acting on the default mode network (DMN). A seminal positron emission tomography (PET) study demonstrated that serotonergic neurotransmission within the DMN plays a pivotal role in its function (e.g., self-referential processing) (Hahn et al. 2012). Of note, this study reports a significant positive correlation between serotonin-1A (5-HT_{1A}) receptor density and DMN functional connectivity. An earlier study suggests that variability in 5-HT_{1A} receptor density results from genetic variations in 5-HTTLPR (David et al. 2005). Despite a conflicting report (Borg et al. 2009), this finding has been replicated in other studies (Christian et al. 2013; Baldinger et al. 2015). Taken together, this literature suggests that genetic variations of the 5-HT transporter gene may underlie variability in DMN connectivity and function.

The DMN and 5-HTTLPR have both been associated with several behavioral traits, but here we focus on impulsivity for the following reasons: First, we have found that familial depression affects connectivity between the DMN and the executive control network (ECN), and this effect on DMN–ECN connectivity is associated with increased impulsivity (Posner et al. 2016). This is consistent with neurocognitive theories suggesting that interactions between the DMN and ECN may index attentional and impulse control. Second, within the same family cohort study, we previously found an association between a family history of depression and low-efficiency variants of 5-HTTLPR (Talati et al. 2015). Taken together, these studies along with the aforementioned relationship between 5-HT and the DMN, led us to hypothesize that DMN connectivity may account, at least in part, for the putative link between 5-HT, familial depression, and impulsivity (Soubrie 1986; Evenden 1999). Specifically, we hypothesized that (1) low transcriptional efficiency 5-HTT genotypes (e.g., 5-HTTLPR and rs25531) would be associated with reduced DMN connectivity and that (2) this genotypic effect on connectivity would mediate an association between 5-HTT genotypes and increased impulsivity. We tested this hypothesis in participants within our family cohort study (Weissman et al. 2016) that was designed to examine differences between families at high and low risk for depression. Risk was defined as the presence or absence of depression in the first generation (G1). Though the original focus of our family study was depression, the children (Generation 2, G2) and grandchildren (Generation 3, G3) have been shown to have increased rates of a range of psychopathology including disorders of impulsivity such as disruptive behavior disorders and substance abuse.

Materials and Methods

Participants

Details on the family depression study are reported elsewhere (Weissman et al. 2016). Briefly, risk status for depression was defined based on the first generation (G1); offspring (Generations 2 and 3, G2 and G3) were defined as high-risk if G1 had a history of major depressive disorder (MDD), and were otherwise defined as low-risk. The current study is based on data collection from G2 and G3. Diagnostic interviews were conducted using the Schedule for Affective Disorders and Schizophrenia–Lifetime Version (the adult version [Spitzer 1979] for participants over age 18 years, and the child version [Kaufman et al. 1997] for participants 6–17 years of age). We obtained diffusion magnetic resonance imaging (dMRI) scans from 94 descendants of G1 families; of them, from 92 participants we collected genetic samples. Exclusion criteria consisted of psychotic symptoms, pregnancy, and MRI contraindications.

MRI Acquisition and Preprocessing

T1-weighted structural (voxel = $1\,m^3$, dimensions = $256 \times 256 \times 162$) and diffusion MRI (voxel = $0.94 \times 0.94 \times 2.5\,mm^3$, dimensions = $256 \times 256 \times 58$, $b = 1000\,sm^{-2}$, number of gradient direction = 15 (Increasing the numbers of diffusion directions may improve the accuracy of fiber estimation in general; however, a study demonstrates reproducibility of tractography dMRI with 12 directions is comparable to one based on 60 directions across the various major white matter pathways (Heriervang et al. 2006). Therefore, we believe 15 directions of b weights in our dMRI suffice to test our hypothesis in DMN white matter connectivity), b0 images = 3) data were acquired on a 3T GE 750 scanner at the New York State Psychiatric Institute. Cortical parcellation based on structural MRI was performed using the recon-all procedure in Freesurfer image data analysis suite (http://freesurfer.net). Diffusion MRI data were preprocessed through a pipeline in Functional MRI of the Brain (FMRIB)'s Diffusion Toolbox (Smith et al. 2004), which includes skull stripping, eddy current correction, subsequent B-matrix rotation, and multi-fiber probabilistic diffusion model fitting. Structural and diffusion MRI modalities were registered to each other using FMRIB's Linear Image Registration Tool (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FLIRT). For the diffusion scans, 2 in-scanner head motion parameters were estimated: average volume-by-volume translation and rotation (Yendiki et al. 2013) (see Table 1).

Regions of Interests definition

We derived "functionally informed anatomical" regions of interests (ROIs) for diffusion tractography; this involves 2 step. First, we defined anatomical masks to account for inter-subject variability in neuroanatomy (e.g., cortical surface shapes) using Freesurfer's cortical parcellation based on the Desikan & Killiany atlas (Desikan et al. 2006). Second, among the Freesurfer parcellated anatomical regions, we then selected the regions most closely associated with a given functional network (e.g., DMN or ECN). For this, we used a resting-state functional network atlas (http://findlab.stanford.edu/functional_ROIs.html) and calculated the extent to which a Freesurfer parcellated region (e.g., the precuneus) overlapped with the DMN or ECN (based on the functional network atlas). From these calculations, we selected the Freesurfer parcellated regions with the greatest overlap with the DMN or ECN to select most relevant anatomical region to a given functional network. In this way, our tractography method is in line with the majority of tractography studies that have used anatomical masks, while at the same time, having potential resting-state functional network correlates. We reasoned that this method was preferable over some alternatives. For example, masks derived purely from an anatomical atlas might be limited in their relationship to resting-state functional networks. Conversely, masks derived directly from a functional atlas

Table 1. Demographic information and in-scanner head motion

	Family history of depression				5-HTTLPR/rs25531				
	High-risk (n = 50)	Low-risk (n = 42)	Test Statistic	P-value	Low/low (n = 8)	Low/high (n = 53)	High/high (n = 31)	Test statistic	P-value
Family risk									
High-risk (n = 50)	—	—	—	—	6	28	16	X^2 = 1.51	0.46
Low-risk (n = 42)	—	—	—	—	2	25	15		
5-HTTLPR/rs25531									
S′ or Lg (n = 61)	34	27	X^2 = 0.14	0.71	—	—	—	—	—
L′ (n = 31)	16	15			—	—	—	—	—
Age, mean	34.9 ± 14.4	29.5 ± 12.9	t = 1.9	0.06	31.4 ± 8.06	32.8 ± 14.17	31.2 ± 14.55	F = 1.38	0.87
Age, by generation									
Second generation (G2)	45.9 ± 8.2	47.6 ± 5.7	t = 0.7	0.49	40.1 ± 3.64	46.6 ± 6.6	47.1 ± 9.41	F = 1.15	0.32
Third generation (G3)	20.9 ± 5.2	21.9 ± 4.6	t = 0.8	0.46	26.2 ± 4.18	20.8 ± 4.81	21.1 ± 4.75	F = 2.75	0.073
Generation									
Second generation (G2)	28	13	X^2 = 5.7	0.02*	3	24	12	X^2 = 0.43	0.81
Third generation (G3)	23	31			5	29	19		
Gender									
Male	21	22	X^2 = 0.5	0.29	3	30	17	X^2 = 1.02	0.59
Female	30	23			5	23	14		
Depressive symptoms									
Adult	3.2 ± 4.7	1.6 ± 4.5	t = 1.5	0.13	2.2 ± 2.62	2.3 ± 4.06	2.7 ± 6.05	F = 0.06	0.93
Child	17.8 ± 0.9	18.8 ± 3.5	t = 0.8	0.45	—	18.3 ± 2.38	17.5 ± 1.00	F = 0.47	0.50
Anxiety symptoms									
Adult	2.7 ± 4.3	1.0 ± 2.9	t = 1.9	0.06	2.5 ± 2.58	1.6 ± 3.75	2.0 ± 4.02	F = 0.14	0.86
Child	3.6 ± 2.6	4.2 ± 4.3	t = 0.3	0.76	—	3.4 ± 3.82	4.4 ± 2.51	F = 0.24	0.63
Current or prior psychotropic medications	8/50	4/42	X^2 = 0.9	0.34	3/8	7/53	2/31	X^2 = 5.408	0.067
Current/lifetime depressive disorder	30/50	12/42	X^2 = 10.1	0.002**	7/8	26/53	8/31	X^2 = 10.8	0.004**
Current/lifetime anxiety disorder	33/50	20/42	X^2 = 4.0	0.046*	8/8	27/53	16/31	X^2 = 7.04	0.29
Current/lifetime substance use disorder	16/50	11/42	X^2 = 0.57	0.45	1/8	19/53	7/31	X^2 = 2.8	0.24
Head motion during dMRI, translation	0.61 ± 0.2641	0.59 ± 0.351	t = −0.21	0.83	0.62 ± 0.189	0.60 ± 0.313	0.61 ± 0.312	F = 0.026	0.97
Head motion during dMRI, rotation	0.005 ± 0.0031	0.005 ± 0.0044	t = 0.150	0.87	0.005 ± 0.0023	0.004 ± 0.0034	0.006 ± 0.0047	F = 0.33	0.71

Note: Depressive symptoms were determined by Hamilton Depression Rating Scale and the Children's Depression Inventory for adults and children, respectively. Anxiety symptoms were determined by the Hamilton Anxiety Rating Scale and the Revised Children's Manifest Anxiety Scale for adults and children, respectively. Values are mean ± SD unless specified. *P < 0.05; **P < 0.01.

(e.g., applying an individual or group resting-state functional network map) would not account for the fact that the structure-function relationship of the brain networks is a one-to-one correspondence: i.e., "functional connections exist between regions with no direct structural connection, and indirect connections and interregional distance accounted for some of the variance in functional connectivity that was unexplained by direct structural connectivity" (Honey et al. 2009).

For the DMN, we divided the DMN functional atlas into 2 anatomically distinct subdivisions, the anterior and posterior DMN (recent literature suggests a functional distinction between these 2 subdivisions [Knyazev 2012] with differential implications to neuropathology [Lehmann et al. 2013]).

Comparing the resting-state functional atlas with the anatomical parcellation, we found that the precuneus had the greatest overlap with the posterior DMN, encompassing 25.7% of the posterior DMN, followed by the posterior cingulate cortex, encompassing 13.9% of this network. The regions with the greatest overlap with the anterior DMN were the medial orbitofrontal cortex (mOFC) (encompassing 50.6% of this network) and the superior frontal gyrus (SFG) (encompassing 33.3% of this network). For the ECN, the SFG and the rostral middle frontal gyrus (rMFG) had the greatest overlap (encompassing 22.2% and 15.4% of the network, respectively). Based on these calculations, we selected the precuneus and mOFC as ROIs for posterior and anterior DMN, respectively, and the SFG and rMFG as ROIs for the ECN (Fig. 1A). In addition to these hypothesized regions, we included the hippocampus as an exploratory target region, given its connectivity with the DMN (Greicius et al. 2009). Our ROI selection is in line with the literature on DMN structural connectivity (reviewed in Cavanna and Trimble 2006).

Given the main focus of this study on the posterior DMN (i.e., our prior findings on the correlation between posterior DMN and impulsivity [Posner et al. 2016] and the literature on the link between the posterior DMN and serotonergic transmission [David et al. 2005; Hahn et al. 2012]), we used the precuneus (posterior DMN) as a seed region for diffusion tractography, and others as target regions.

Functional networks are not mutually exclusive with each other in terms of anatomical delineation: for example, the precuneus constituted the largest (25.7%) portion of the posterior DMN, 3.3% of the ECN, and 3.1% of the salience network. Furthermore, unlike functional connectivity, white matter tracts may not account for indirect connections among regions. Therefore, as noted elsewhere (Honey et al. 2009), structural connectivity may not correspond directly with functional connectivity. Rather, structural connectivity is deemed to be an important constraint to functional connectivity. This point should be considered when interpreting the results.

Diffusion Probabilistic Tractography

We estimated the strength of white matter tracts starting from the DMN using probabilistic tracking with crossing fibers (two-fiber model; probtrackx2 in FSL; Behrens et al. 2003). To improve reliability of our probabilistic tractography results, we increased the number of iterations from 5000 (default value) to 25 000. To improve sensitivity (i.e., true positive rate), we dilated masks using fslmaths program in FSL (using a kernel method); this was meant to increase the number of tracts estimated in the white matter space surrounding a given mask. Lastly, to improve specificity (i.e., true negative rate), we eliminated false positive tracts using a stopping mask for cerebral spinal fluid and dura matter.

Target masks (see ROI definition above) were used to calculate the numbers of streamlines, but did not constrain the tractography procedures. We estimated relative connectivity strength (Forstmann et al. 2012; Chowdhury et al. 2013; Cha et al. 2015) of the DMN (i.e., either within DMN or between DMN and ECN) based on the number of streamlines reaching target ROIs in proportion to the total numbers of streamlines (i.e., all streamlines from the precuneus):

$$\text{Connectivity}(p)\ \text{precuneus} \sim \text{target} = \frac{\#\ \text{of streamlines between precuneus} \sim \text{target}}{\#\ \text{of streamlines from the precuneus}}$$

Therefore, our connectivity measures represent relative, region-to-region, connection probability.

To validate the results from our local tractography method, we additionally performed a global tractography analysis. Using a fully automated probabilistic tractography approach, Tracts Constrained by Underlying Anatomy, or TRACULA (Yendiki et al. 2011), we reconstructed the cingulum, the major fascicle most likely connecting the posterior DMN and the ECN.

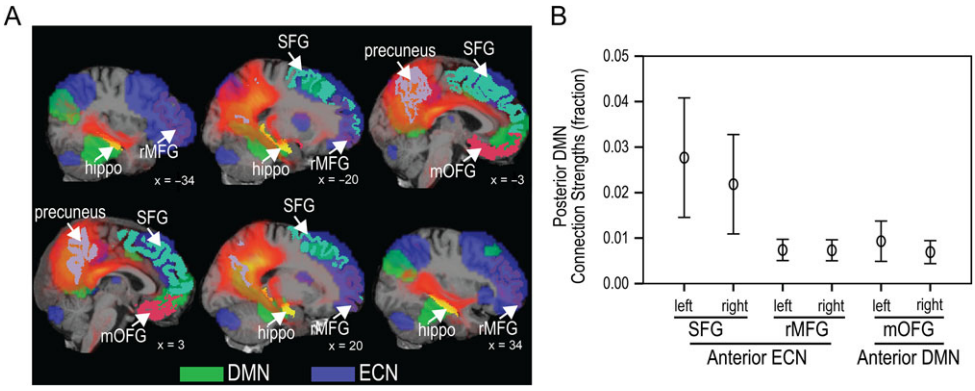

Figure 1. White matter connectivity of DMN. (*A*) A probability map (red-yellow) of white matter tracts from the posterior DMN seed (the precuneus; shown in light blue; see Materials and Methods section for details) is shown based on probabilistic tractography averaged across subjects. Overlaid are masks to estimate structural connection strengths: the SFG (shown in jade) and rMFG (shown in purple) for the anterior DMN (shown in green); and the mOFG (shown in red) for the executive control network network (ECN; shown in blue). The functional network masks were derived from an atlas (http://findlab.stanford.edu/functional_ROIs.html). Based on calculations of the overlap between each functional atlas and each anatomical mask, these masks were the most representative of each functional network (see Materials and Methods section for details). Masks were registered to and overlaid with a Montreal Neurological Institute 152 T1 structural image. (*B*) Mean connection strengths of posterior DMN across the frontal masks. Hippo, hippocampus. Error bars indicate 95% confidence interval.

For statistical analysis, we used mean factional anisotropy (FA), a summary measure of microstructural integrity of the white matter. Therefore, our tractography analyses present complementary methodologies combining 2 probabilistic tractography methods (local and global) and combining 2 measures of white matter connectivity (streamline-based and anisotropic measurements).

Genotyping

DNA was extracted from saliva collected using Oragene DNA Self Collection Kit following standard manufacturer protocol (Oragene Genotek). The region encompassing 5-HTTLPR and rs25531 polymorphisms was amplified with primers; FORWARD: 5′TCCTCCGCTTTGGCGCCTCTTCC-3′; REVERSE: 5′-TGGGGGTTGCAGGGGAGATCCTG-3′ via a polymerase chain reaction in multiplex master mix (Qiagen). Amplicon was resolved on a 2.3% UltraPure™ Agarose (Invitrogen), and visualized under the UV transilluminator. Here, 512 bp and 469 bp bands were called as L and S allele at 5-HTTLPR, respectively. For rs25531, amplicon was digested with restriction endonuclease MspI (New England Biolabs® Inc.), and the product resolved in a 2.9% UltraPure Agarose (Invitrogen) and visualized under the UV transilluminator. Digested fragments of 402 bp were labeled G at rs25531. Parallel analysis of amplicon and restriction fragment products allowed us to determine a phase of the 5-HTTLPR/rs25531 haplotype in each individual. Genotype labeling was blind to subject familial risk group.

The triallelic polymorphisms (S/La/Lg) were recoded as biallelic because the transcriptional efficiency of Lg is similar to that of the S allele (Hu et al. 2006). Three genotypes were considered based on high (La) versus low transcriptional efficiency (Sa, Sg, or Lg): low/low, low/high, or high/high. Ninety-two participants' genetic samples were collected and assayed.

Neuropsychological and Symptom Assessment

Impulsivity was assessed with the Conner's Continuous Performance Task II (CPT-II) (Conners and Staff 2000). Briefly, in the CPT-II, participants are instructed to hit a space bar whenever a letter appears on the computer screen, but to not hit the space bar when the letter "X" is shown. Commission errors are made when a participant hits the space bar when the "X" is shown. In addition, omission errors, variability, and perseveration scores were collected. Normed scores (by age and sex) were used. Depressive symptoms were assessed by Hamilton Depression Rating Scale (24 items) (Hamilton 1960) and the Children's Depression Inventory (27 items) (Kovacs 1992) for adults and children, respectively. Anxiety symptoms were assessed by the Hamilton Anxiety Rating Scale (14 items) (Maier et al. 1988) and the Revised Children's Manifest Anxiety Scale (37 items) (Reynolds and Richmond 1985) for adults and children, respectively. A complete list of the study questionnaires is provided online: http://www.highriskdepression.org.

Statistical Analysis

We examined associations between DMN connectivity and 5-HTTLPR/rs25531 using generalized estimating equations (GEEs) in GWAF (Genome-Wide Association analysis with Family data) R-package (Chen and Yang 2010). This method handles relatedness of samples using an independence working correlation matrix with each family modeled as a cluster in a robust variance estimate for genotype effects. In a GEE model, DMN connectivity was used as the dependent variable (DV); genotypes and familial history of depression as the independent variables; a genotype-by-familial history interaction, and potential confounding variables (age, sex, current depressive symptoms, current anxiety symptoms, history of depressive or anxiety disorder, history of substance abuse disorder, medications for past 3 months, and 2 in-scanner head motion parameters). Significance was corrected against 6 tests (for 6 target regions: SFG, rMFG, and mOFC in each hemisphere) using the false-discovery rate (FDR; Benjamini and Hochberg 1995). Effect size was estimated using a pseudo-r^2 method for linear mixed-effect models (Nakagawa and Schielzeth 2013) available in R-package (https://cran.r-project.org/web/packages/MuMIn). As a control analysis, we tested an effect of genotypes on cortical morphometry (i.e., thickness) of the seed/target regions of which connectivity showed a significant effect of genotypes.

Second, we examined associations between 5-HTTLPR/rs25531 and behavioral measures of impulsivity (CPT-II) using GWAF. Similar GEE models were used as above except for CPT-II was used as the DV. Significance was corrected using FDR for the 4 behavioral outcomes assessed by the CPT-II. Effects adjusted for the following covariates: age, sex, current depressive symptoms, current anxiety symptoms, history of depressive or anxiety disorder, history of substance abuse disorder, medications for past 3 months.

Lastly, we tested a hypothesis that DMN connectivity mediates the associate between 5-HTTLPR/rs25531 and impulsivity using a mediation analysis. To account for nonindependence of samples due to familial relatedness, we entered family (familial ID) as a random variable using R-package of linear mixed-effects model (Bates et al. 2015) (https://cran.r-project.org/web/packages/lme4). Using "Mediation" R-package (Tingley et al. 2014) (https://cran.r-project.org/web/packages/mediation), average causal mediation effects (ACMEs) and average direct effects (ADEs) were assessed. Estimation of ACME and ADE adjusted for family history of depression, genotype-by-familial history interaction, and the confounding variables mentioned above for neural and behavioral measures, respectively. Significance was determined using quasi-Bayesian Monte Carlo simulation with 1000 iterations.

Results

Demographics

The sample consisted of 92 subjects (45 females, 47 males) with a mean age of 34 ± 14.7 (range: 10–68 years old). The distributions of genotypes of 5-HTTLPR/rs25531 did not differ across familial risk groups ($X^2 = 0.14$, $P = 0.71$; Table 1). The rates of current or lifetime history of depression were greater in low transcriptional genotype (i.e., low/low and low/high) carriers-of 5-HTTLPR/rs25531 compared with La/La genotype carriers ($X^2 = 6.7$; $P = 0.01$). In Hardy–Weinberg equilibrium, we found no evidence that distributions of 5-HTTLPR ($P = 0.053$) or rs25531 ($P = 0.503$) deviated from the expected frequencies (we are not concerned by the rather low P-value for the 5-HTTLPR distributions, because the false positive rate of the Hardy–Weinberg equilibrium in this family study is expected to be inflated due to the relatedness of samples [Bourgain et al. 2004]).

Characterization of DMN White Matter Tracts

Probabilistic tractography estimated the white matter tracts from the precuneus to the frontal lobes (Fig. 1A). The tracts from the precuneus traversed the cingulum reaching the

medial orbital frontal gyrus (mOFG) (a node within the anterior DMN) and the SFG and rMFG (nodes within the ECN). Another significant portion the precuneus-seeded tracts extended to the temporal lobes terminating in the hippocampus (a node within the DMN). Among the target ROIs, the SFG showed the strongest connectivity with the precuneus (Fig. 1B).

Association Between 5-HTTLPR/rs25531 and DMN Connectivity

Testing our first hypothesis regarding associations between the 5-HTT genetic variants and DMN connectivity, we found a significant effect of low transcriptional efficiency genotypes on precuneus-SFG white matter connectivity. Low-efficiency genotypes were associated significantly with a decrease in precuneus-SFG connectivity in the left hemisphere (beta = 0.012, P_{FDR} = 0.012; Fig. 2A) with 9.4% of the variance accounted by the genotype (pseudo-r^2 for linear mixed-effect models) (Nakagawa and Schielzeth 2013), but not in the right hemisphere ($P > 0.6$) (an exploratory analysis on the posterior cingulate cortex-seeded white matter tracts to the SFG showed no significant effect of the genotypes [P's > 0.13]). Post hoc group-wise comparison showed a dose-dependent effect of low-efficiency genotypes. The effect adjusted for covariates (see Materials and Methods section). The effect of genotype remained significant even after excluding participants with a history of prior exposure of psychotropic medication or a history of a substance abuse disorder ($P = 0.0025$), both of which are potential confounds influencing the 5-HT system. We found no significant associations between the genotypes and precuneus or SFG gray matter morphology (e.g., thickness) (P's > 0.12), suggesting specificity of the association between genotype and brain connectivity. Genotype-by-familial risk interaction did not reach significance after correction for multiple comparisons (P_{FDR} = 0.096). For the other ROIs (i.e., precuneus-mOFC, likely related to posterior–anterior DMN connection, and precuneus-rMFG, likely related to DMN–ECN connection) the precuneus connectivity showed nonsignificant effects for Genotype, Familial risk, or Genotype-by-Familial risk (P's > 0.2).

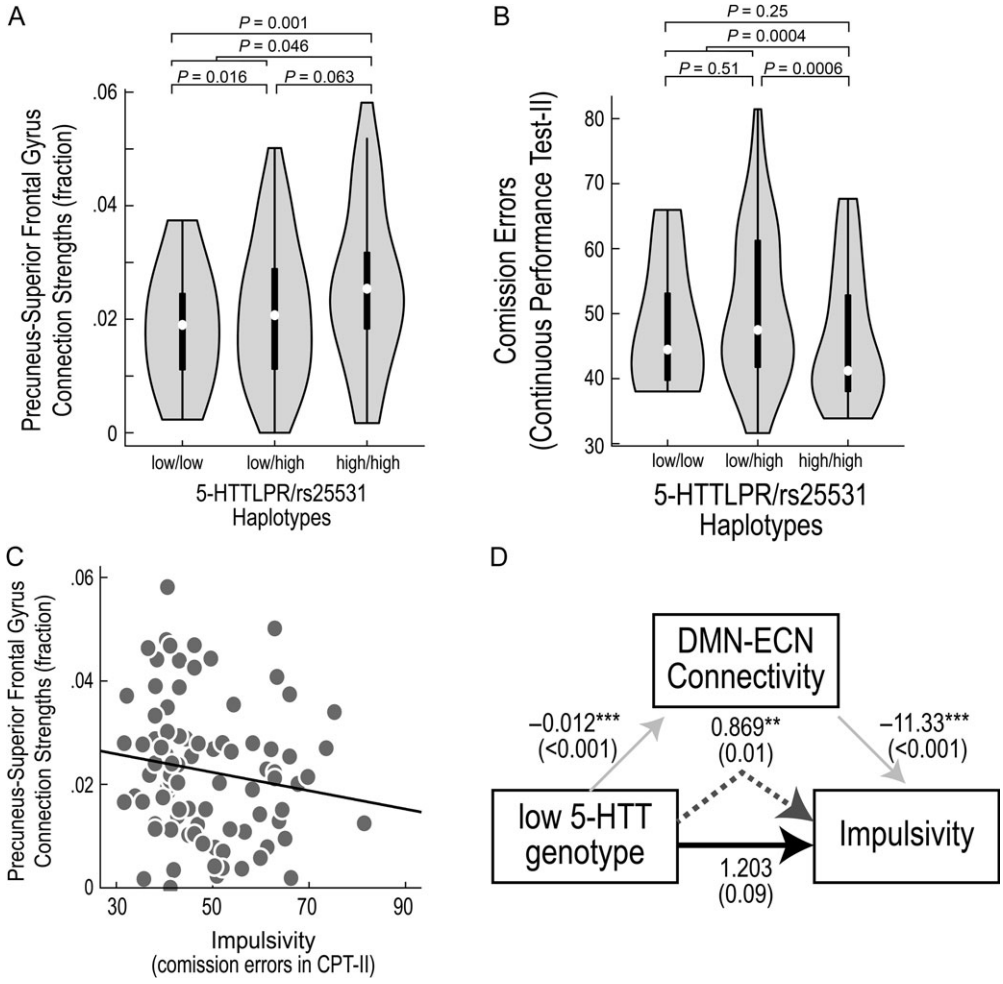

Figure 2. Associations of genetic variations of serotonin transporter (5-HTT), DMN connectivity, and impulsivity. (*A*) A box plot shows that low transcriptional efficiency genotypes of 5-HTTLPR/rs25531 are correlated with a decrease in white matter connectivity between posterior DMN and the SFG (P_{FDR} = 0.012). Significance of post hoc group-wise comparisons are shown. Significant effects were detected for either the low/low group ($P = 0.001$) or the low/low and low/high groups ($P = 0.046$) relative to the high/high group. (*B*) Low-efficiency genotypes correlated with an increase in a neuropsychological measure of impulsivity (commission error scores in CPT-II). Significance of post hoc comparisons are shown. Significant effects were detected for either the low/high group ($P = 0.004$) or the low/low and low/high groups ($P = 0.0006$) relative to the high/high group. (*C*) A scatter plot shows an association between decreased posterior DMN connectivity and increased impulsivity ($P = 0.011$). (*D*) Mediation analysis shows that a decrease in DMN connectivity significantly mediates effects of low 5-HTT efficiency genotypes on increased impulsivity (measured by CPT-II commission errors). The dotted line represents ACME; the solid line, ADE (Tingley et al. 2014).

Because the precuneus-SFG pathway traversed the cingulum, we further tested the effects of 5-HTTLPR/rs25531 on fiber integrity within the cingulum. GWAF analysis showed that the low-efficiency genotypes were associated with a decrease in FA of the left cingulum (beta = 0.016, $P = 0.029$). In the right cingulum, we found nonsignificant effects ($P = 0.67$). The mean FA of the left cingulum was significantly correlated with the probabilistic tractography measures of precuneus-SFG connectivity ($r = 0.277$, $P = 0.008$). These findings present converging evidence for the association between low-efficiency genotypes and decreased DMN (precuneus-SFG) structural connectivity via the cingulum.

Given the prior reports of aberrant connectivity between the DMN and the hippocampus in depression (Sheline et al. 2009; Rocca et al. 2015), we explored relationships between 5-HTTLPR/rs25531 and precuneus-hippocampus white matter connectivity. We found low-efficiency genotypes were associated with decreased precuneus-hippocampus white matter connectivity (beta = 0.007, $P = 0.034$; uncorrected) in the left hemisphere, but not in the right hemisphere ($P > 0.3$). This effect did not survive corrections for multiple comparisons (for hemisphere). Nevertheless, this result leaves open the possibility that 5-HTTLPR/rs25531 affects other parts of the DMN.

Given the wide age range of the participants (12–60 years old), nonlinear maturation of white matter could confound the effects of 5-HTTLPR/rs25531 on left precuneus-SFG connectivity. To examine this, we ran separate GWAF analyses in younger (under 25 years old; $n = 39$) and older (over 25 years old; $n = 53$) participants, respectively. We found similar effects of 5-HTTLPR/rs25531 on left precuneus-SFG connectivity across the 2 age groups (the older group: beta = 0.012, $P = 0.006$; the younger group: beta = 0.013, $P = 0.012$). In sum, significant genotype–connectivity associations were present across developmental stages.

Association Between 5-HTTLPR/rs25531 and Impulsivity

GWAF analysis showed a significant association between 5-HTTLPR/rs25531 and CPT-II, commission score (beta = −7.06, $P_{FDR} = 0.032$; Fig. 2B). In post hoc group-wise comparisons, the contrast of low genotypes carriers (i.e., low/low or low/high) versus high/high genotypes was highly significant ($P = 0.0004$). However, we did not find evidence for a dose-dependent effect. The effects were adjusted for covariates (see Materials and Methods section). Overall, these results reflect increased impulsivity in low-efficiency genotypes carriers.

Path Modeling Between 5-HTTLPR/rs25531, DMN Connectivity, and Impulsivity

We tested our main hypothesis that decreased DMN white matter connectivity mediates the association between 5-HTTLPR/rs25531 and increased impulsivity. Mediation analysis showed that the left precuneus-SFG white matter connectivity mediated significantly the effects of low 5-HTT efficiency genotypes (5-HTTLPR/rs25531) on increased impulsivity (CPT-II commission score) (ACME = 0.869; $P = 0.01$; significance was determined using the Quasi-Bayesian Monte Carlo simulation; [Tingley et al. 2014]) (Fig. 2D). ADE was 1.203 ($P = 0.09$). This model also showed a significant association between DMN connectivity and impulsivity ($b = -11.33$, $P = 0.001$) (Fig. 2C).

Discussion

In this study, we found significant associations across 3 variables of interest: genetic variants of 5-HTT, DMN connectivity, and impulsivity. Path modeling of these 3 variables supported a potential mechanistic account—that is, decreased DMN structural connectivity may instantiate a potential mechanism (i.e., mediation) by which low transcriptional efficiency genotypes contribute to increased impulsivity. Reduced DMN connectivity may, therefore, offer an endophenotype of 5-HTTLPR by which the low-efficiency genotypes predispose for a range of neuropsychiatric disorders by reducing impulsive control. This transdiagnostic interpretation is in line with associations between low transcriptional 5-HTT genetic variants and a wide array of neuropsychiatric disorders (Caspi et al. 2003; Hariri and Holmes 2006; Karg et al. 2011) including those related to impulsivity, such as ADHD (Manor et al. 2001) and substance abuse (Kreek et al. 2005) .

This study contributes to the current literature with novel findings regarding the role of the serotonergic system both in the DMN and in impulse control. In line with an earlier report of the role of the serotonin neurotransmission in DMN (David et al. 2005), our study presents structural evidence of that association, using diffusion probabilistic tractography. Prior functional MRI studies have shown associations between DMN connectivity and psychopathology such as depression (Jacobs et al. 2014), schizophrenia (Whitfield-Gabrieli et al. 2009), bipolar disorder (Chai et al. 2011), and ADHD (Castellanos et al. 2008). Extending this literature, our study suggests the 5-HTT genetic variants may underlie variability in DMN connectivity.

Our results were cross-validated using 2 different tractography methods (i.e., global and local tractography) and 2 different white matter indices (i.e., probabilistic connectivity and white matter anisotropy). Primate tracing studies have revealed that the cingulum carries reciprocal connections between the posterior DMN and the frontal lobes (most strongly with the dorsolateral prefrontal cortex, BA46, which includes the SFG in this study) (Parvizi et al. 2006; Leech and Sharp 2014). As reproducibility is a crucial issue in human genetics (Bosker et al. 2011) and neuroimaging studies (Button et al. 2013), the fact that our tractography findings were robust to methodological variance (global vs. local tractography) is noteworthy. Furthermore, as one of the first diffusion tractography studies reporting an association between DMN white matter connectivity and a behavioral measure of impulsivity, our findings underscore the utility of diffusion tractography in examining DMN connectivity.

It is interesting to note that the association between 5-HTT genetic variants and DMN connectivity is specific to the left, but not right, hemisphere. However, as we have reported elsewhere, family history has a converse pattern based on functional connectivity, such that a positive family history of depression correlates with alterations in right hemispheric DMN–ECN functional connectivity (Posner et al. 2016). Similar findings of contralateral effects for genetic versus environmental influences have been reported elsewhere. For example, a large-scale youth twin study showed lateralized genetic and environmental influences—the genetics influences on brain structure were greater in the left hemisphere, whereas environmental influences were greater on right (Yoon et al. 2010). The association between 5-HTT genetic variants and left hemispheric DMN connectivity is also consistent with a recent PET study reporting that 5-HT(1A) receptor binding is higher in the left versus right hemisphere (Fink et al. 2009). Although firm conclusions about lateralized effects of genetic versus

environmental influences cannot be made on the basis this study, future research may investigate associations between lateralization, DMN connectivity, and psychopathology.

Our path analysis presents a potential gene-brain-behavior pathway by supporting the hypothesis that the association between of 5-HTTLPR/rs25531 and impulsivity is mediated by DMN connectivity. Prior studies (including a meta-analysis) have primarily focused on the relationship between 5-HTTLPR/rs25531 and diagnostic outcomes (e.g., MDD) (Risch et al. 2009) and have yielded inconsistent results. In contrast, we focused on associations between genotype and a neural circuit. This circuit-based approach is transdiagnostic in nature, and congruent with the National Institute of Mental Health (NIMH) Research Domain Criteria (RDoC) initiative, may circumvent incongruities between DSM nosology, on the one hand, and brain topology, on the other.

Previous studies have implicated gene-by-environment interactions between 5-HTTLPR genotypes and life stress on the development of depression (Caspi et al. 2003). In this study, because of limited assessments of early life stress, we were not able to directly test this possibility. However, a related question that we were able to examine was whether family history interacts with 5-HTTLPR genotypes. Here, we found no significant interactions. This finding, however, does not exclude the possibility of gene-by-environment interactions and is limited because family history encompasses both genetic and environmental factors. Moreover, it remains untested whether other brain circuits (e.g., the amygdala-prefrontal circuit) could mediate 5-HTTLPR-by-life stress interactions.

Our assessments of DMN connectivity and CPT-based impulsivity measures convey somewhat different pictures. Regarding DMN connectivity, the low activity genotype was associated with a decreased in structural connectivity in a dose-dependent manner (additive effect), however, for the behavioral measures of impulsivity, no such an additive effect was found in the low/low group (although low genotype carriers had decreased impulsivity compared with the high/high group). While it would require more balanced sample sizes across the genotypes to draw a firm conclusion, some ideas about this seemingly discrepant result are worth consideration. One interpretation is that the genotype–behavior relationship is more complex than is the genotype–connectivity relationship. This is consistent with mixed reports on the genotypic–behavior relationship. For example, one study has shown an association between the short allele and greater impulsivity measured by CPT tests (Walderhaug et al. 2010), whereas another report suggests that the long allele is associated with greater impulsivity on the Go/Nogo task (a task very similar to the CPT) (Nomura et al. 2015). Our path analysis also suggests that whereas DMN connectivity is a mediator of the relationship between genotype and impulsivity, it is unlikely to be the sole mediator. Other factors, such as frustration tolerance and/or amygdala reactivity, may also influence CPT performance and similarly may be influenced by 5-HTTLPR.

Furthermore, the genetic effects on connectivity may also represent a compensatory mechanism. For example, low/low genotype carriers may be more prone to externalizing behaviors (e.g., impulsivity) than other genotype carriers, and this behavioral vulnerability could in turn affect DMN connectivity. This might be analogous to reports of greater activation of the ventromedial prefrontal cortex in short 5-HTTLPR allele carriers to compensate for the effects of exaggerated amygdala responses (Heinz et al. 2005; Pezawas et al. 2005). Further investigation is required to tease apart the direction of causality linking low activity genotypes, DMN connectivity, and impulsivity.

Given the well-known issue of poor reproducibility for imaging-genetics studies, it is worth discussing limitations of this study. First, caution is always warranted when interpreting an effect from a small sample; however, it should be noted that the overall sample size of this study is relatively large compared with other imaging-genetics studies (it is the second largest among the studies listed in a recent meta-analysis of neuroimaging studies of 5-HTTLPR [Murphy et al. 2013]). Also, the association between 5-HTTLPR and DMN connectivity is not only driven by low/low genotypes (the smallest number), but also low/high and high/high genotypes. Moreover, our study replicates earlier functional neuroimaging findings of the association between 5-HTTLPR and posterior DMN–SFG connectivity (Wiggins et al. 2012). Second, the current findings are based on a sample that is largely Caucasian; it remains to be tested whether these findings can be replicated across races and ethnicities. Third, although we attempted to minimize modeling errors of probabilistic tractography by increasing the sampling from 5000 to 25 000 iterations, our tractography results might be further validated using recently developed methods such as linear fascicle evaluation (Pestilli et al. 2014) or spherical-deconvolution informed filtering of tractograms (SIFT) (Smith et al. 2013).

In conclusion, this study builds upon our previous work associating low-efficiency 5-HTTLPR/rs25531 genotypes with a family history of depression (Talati et al. 2015). Here, we found associations between 5-HTTLPR/rs25531 genotypes, DMN connectivity, and impulsivity. These findings may reflect a potential pathway by which risk for psychopathology is inherited and suggest potential targets for novel treatments.

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Notes

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