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EPISTATIC INTERACTION OF BDNF AND COMT ON THE FRONTOSTRIATAL SYSTEM

C. WANG,[a†] B. LIU,[b,c†] H. LONG,[b,c] L. FAN,[b] J. LI,[b] X. ZHANG,[b] C. QIU,[b] C. YU[e*] AND T. JIANG[a,b,c,d,f*]

[a] *Key Laboratory for NeuroInformation of Ministry of Education, School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu, China*

[b] *Brainnetome Center, Institute of Automation, Chinese Academy of Sciences, Beijing, China*

[c] *National Laboratory of Pattern Recognition, Institute of Automation, Chinese Academy of Sciences, Beijing, China*

[d] *CAS Center for Excellence in Brain Science, Institute of Automation, Chinese Academy of Sciences, Beijing, China*

[e] *Department of Radiology, Tianjin Medical University General Hospital, Tianjin, China*

[f] *The Queensland Brain Institute, University of Queensland, Brisbane, Australia*

Abstract—The frontostriatal system plays a critical role in emotional and cognitive control. Brain-derived neurotrophic factor (BDNF) influences the release of dopamine (DA) in the ventral striatum (VST), while catechol-O-methyltransferase (COMT) impacts DA availability in the prefrontal cortex (PFC). Behavioral studies have already shown a genetic interaction of BDNF Val66Met and COMT Val158Met, but the interaction on the DA-related neural circuit has not been previously studied. Here we show, using functional magnetic resonance imaging in a sample of healthy human subjects, that BDNF and COMT epistatically interacted on the functional connectivity between the bilateral VST and the anterior cingulate cortex. Specifically, BDNF Val66Met impacted the VST-PFC functional connectivity in an inverted U-shaped in COMT Met carriers, while COMT Val homozygotes displayed a U-shaped. These data may be helpful elucidating the mechanism of the interaction between BDNF and COMT on the cognitive functions that are based in the frontostriatal system.

Key words: brain-derived neurotrophic factor (BDNF), catechol-O-methyltransferase (COMT), dopamine, frontostriatal system, functional connectivity.

INTRODUCTION

Dopamine (DA) may mediate prefrontal cortex (PFC) functions by targeting the frontostriatal circuit, which begins in the PFC and projects to the striatum (Alexander et al., 1986; Tekin and Cummings, 2002; Haber et al., 2006). The fronto striatal circuit is critical for reward processing, emotional regulation, learning and cognitive control (Cardinal et al., 2002; Galvan et al., 2005; Chudasama and Robbins, 2006). Consequently, animals and patients, with lesioned or dysfunction of the frontostriatal pathway, have impairments on prefrontally dependent learning, memory and reward performance (Meyer-Lindenberg et al., 2007; Heller et al., 2009). And the abnormalities are characteristic of the emotional and cognitive deficits found in conditions such as schizophrenia, depression and drug addiction (Alexopoulos, 2002; Harrison et al., 2009; Ersche et al., 2011).

The dopaminergic functions in the frontostriatal circuit are controlled by two important proteins: brain-derived neurotrophic factor (BDNF) and catechol-O-methyltransferase (COMT). BDNF influences the release of DA in the nucleus accumbens (NAc), more broadly, the ventral striatum (VST) (Goggi et al., 2002; Eisch et al., 2003; Narita et al., 2003; Berton et al., 2006); while COMT acts as an enzyme involved in regulating DA degradation and availability in the PFC (Meyer-Lindenberg et al., 2005). The PFC and VST are the initial and terminal regions of the frontostriatal circuit, respectively, so these two proteins interact to regulate the DA level of this circuit.

In humans, both BDNF and COMT genes have functional polymorphisms that code for a substitution of valine by methionine. BDNF Val66Met affects intracellular processing and activity-dependent secretion of BDNF (Chen et al., 2004b); whereas COMT Val158Met causes variations in COMT enzymatic activity (Chen et al., 2004a). Prior studies have documented that these two polymorphisms were involved in the PFC and in striatal dependent function and structure (Egan et al., 2001; Pezawas et al., 2004; Pecina et al., 2014). The

*Correspondence to: C. Yu, Department of Radiology, Tianjin Medical University General Hospital, No. 154, Anshan Road, Heping District, Tianjin 300052, China. Fax: +86-22-6036-2990 or T. Jiang, Brainnetome Center, Institute of Automation, Chinese Academy of Sciences, Beijing 100190, China. Tel: +86-10-8254-4778; fax: +86-10-8254-4777.

E-mail addresses: chunshuiyu@vip.163.com (C. Yu), jiangtz@nlpr.ia.ac.cn (T. Jiang).

† Chao Wang and Bing Liu contributed equally to this work and should be considered co-first authors.

Abbreviations: ACC, anterior cingulate; ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; COMT, catechol-O-methyltransferase; DA, dopamine; fMRI, functional magnetic resonance imaging; FWE, family-wise-error; MANCOVA, multivariate analysis of covariance; MNI, Montreal Neurological Institute; NAc, nucleus accumbens; PCR, polymerase chain reaction; PFC, prefrontal cortex; rs-FC, resting-state functional connectivity; SPM, statistical parametric mapping; SVC, small-volume correction; VST, ventral striatum.

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COMT Val158Met polymorphism has consistently been associated with activity and functional connectivity of PFC during executive function tasks, especially working memory (Tunbridge et al., 2013). Furthermore, it also impacts the activation of subcortical regions including amygdala and VST during emotional processing tasks (Drabant et al., 2006). Other functional magnetic resonance imaging (fMRI) studies have also found activation changes in ventral medial PFC influenced by BDNF Val66Met during memory extinction and resting state (Soliman et al., 2010). Moreover, behavioral research has previously suggested potential interactions between BDNF and COMT in cognitive processes that depend on the integrity of the frontostriatal circuit (Nagel et al., 2008; Witte et al., 2012), but the mechanism of this genetic interaction in the frontostriatal circuit remains unclear.

fMRI measures brain activity by assessing changes in blood flow *in vivo*, and resting-state functional connectivity (rs-FC) provides a means for detecting correlations in spontaneous neural activity between separated brain regions implicated in a functional circuit or network (Fox et al., 2005; Fox and Raichle, 2007) and minimizes the confounds encountered by having the participants perform a variety of specific tasks when assessing spontaneous fluctuations (Power et al., 2011).

How BDNF Val66Met and COMT Val158Met interact to affect the DA-related frontostriatal circuit has not previously been studied. Hence, the present study utilized the bilateral VST as seed regions. Moreover, rs-FC, a non-invasive measure of changes in neuronal baseline activity, was used to study the impact of BDNF Val66Met and COMT Val158Met on the functional connectivity between the VST and the PFC.

EXPERIMENTAL PROCEDURES

Subjects

Three hundred and twenty-three healthy, young Chinese subjects (157 males and 166 females, mean age = 22.7 years, range = 18–31 years) were initially recruited via advertisement. Written informed consent was obtained from all subjects; the study was approved by the local Medical Research Ethics Committee of Tianjin Medical University. The exclusion criteria were reported in our previous study (Wang et al., 2014). We carefully interviewed each of the subjects to ensure that they had no personal or family history of neurological or psychiatric disease, head injury, psychiatric treatment, drug or alcohol abuse, hypothyroidism or other mental diseases and no contraindications to magnetic resonance imaging (MRI) screening. And we used the Chinese Revised Wechsler Adult Intelligence Scale (WAIS-RC) (Gong, 1982) to examine the subjects. Fifty-seven subjects were discarded in the fMRI analysis due to missing genotype data or poor imaging quality. All participants were right-handed and Han Chinese (for demographic details, see Table 1).

DNA extraction and BDNF Val66Met genotyping

DNA was extracted from whole blood using EZgene™ Blood gDNA Miniprep Kit (Biomiga Inc., San Diego, CA, United States of America). BDNF Val66Met and COMT Val158Met were genotyped in all subjects using polymerase chain reaction (PCR) and the ligation detection reaction (LDR) method (Thomas et al., 2004; Yi et al., 2009). The PCR primer sequences and probes of BDNF and COMT were designed as described in our previous studies (Liu et al., 2010; Wang et al., 2014). Due to missing BDNF and COMT genotype data, 29 subjects were excluded from the subsequent analyses. A chi-square test was used to test whether the allele frequencies of BDNF Val66Met and COMT Val158Met fell within the Hardy–Weinberg equilibrium.

Magnetic resonance imaging acquisition and analysis

MRI data were acquired with a single 3-T GE scanner (SIGNAHDX3.0 T scanner; GE Healthcare; Milwaukee, WI, USA), equipped with a standard head coil. Functional images were acquired with a single-shot, T2∗-weighted gradient echo, echo-planar-imaging sequence (TR = 2000 ms, TE = 30 ms, no gap, voxel size = 3.75 mm × 3.75 mm × 4.0 mm, FOV = 240 × 240 mm^2, matrix = 64 × 64, flip angle = 90°, 40 slices, 180 volumes). All subjects were told to relax, lie still with their eyes closed, and not to fall asleep. We further asked whether they had fallen asleep or not during and after the scanning.

Table 1. Between-genotype comparisons of demographic data

COMT	Met carriers			Val/Val		
BDNF	Val/Val	Val/Met	Met/Met	Val/Val	Val/Met	Met/Met
Number	43	66	27	40	69	21
Age[a] (SD)	23.63	22.24	22.33	22.90	23.07	22.43
	(2.60)	(2.11)	(2.50)	(2.61)	(2.45)	(2.25)
Male/female[b,c]	21/22	27/39	16/11	18/22	32/37	6/15
Education[a] (SD)	16.10	15.07	14.54	15.97	16.01	15.68
	(2.09)	(2.90)	(3.10)	(1.92)	(2.04)	(3.65)
IQ[a] (SD)	115.69	117.18	114.63	118.30	118.04	118.05
	(10.86)	(8.26)	(10.15)	(6.58)	(9.44)	(7.05)

SD, standard deviation.
[a] Two-way ANOVA ($p > 0.05$).
[b] Pearson Chi-square test on BDNF ($p > 0.05$).
[c] Pearson Chi-square test on COMT ($p > 0.05$).

All the raw fMRI data were inspected by two experienced radiologists who knew nothing about the genotype information. Fifteen subjects were excluded because of poor imaging quality, such as apparent signal loss and inter-slice motion artifacts. All the preprocessing and statistical steps were completed using statistical parametric mapping (SPM8, http://www.fil.ion.ucl.ac.uk/spm). The preprocessing steps, including discarding the first 10 volumes of each scan slice timing, head motion correction, spatially normalizing to the Montreal Neurological Institute (MNI) template, resampling to $2 \times 2 \times 2\ mm^3$, smoothing with a 4-mm Gaussian kernel, temporal band-pass filtering, and regressing out nuisance signals including head motion parameters and white matter, cerebrospinal fluid and global signals, were performed using DPARSFA (Data Processing Assistant for Resting-State fMRI Advanced Edition, http://www.restfmri.net/forum/DPARSF) in SPM. A further 13 participants who exhibited a maximum displacement in any of the cardinal directions (x, y, z) of greater than 2 mm or a maximum spin (x, y, z) of greater than 2° were excluded. In the end, 266 subjects were included in the functional connectivity analysis.

We extracted left and right VST seed regions from the Oxford-GSK-Imanova Structural-anatomical Striatal Atlas (Tziortzi et al., 2011) and resampled the masks for the seed regions to $2 \times 2 \times 2\ mm^3$ in MNI space. The mean time series for each seed region were obtained for each subject. Then, Pearson correlation coefficients between the mean time series of the seed regions and the rest of the whole brain were computed in a voxel-wise manner. Fisher's r-to-z transformation was subsequently used to convert the correlation coefficients to z values. To identify the brain regions that showed significant functional connectivity with the seed regions, we conducted one-sample t-tests on the individuals' z-FC maps ($p < 0.05$, family-wise-error (FWE) correction) with age and sex as covariates.

The comparisons of the z-FC maps were performed using a two-way (BDNF genotype and COMT genotype) analysis of variance (ANOVA) with age and sex as covariates. To test the hypothesis that BDNF and COMT have an interactive effect on the VST-PFC pathway, we restricted to the results within the PFC which combined the BA9, BA10, BA11, BA24, BA25, BA32, BA44, BA45, BA46 and BA47 regions. Then we averaged the z-scores of the significant clusters for all subjects, a multivariate analysis of covariance (MANCOVA) and a post hoc MANCOVA and post hoc t-test were used to examine the single and interactive effects of COMT and BDNF genotypes on the z-scores of the significant clusters. The alpha level was adjusted (Bonferroni) for multiple comparisons. Analyses were performed using SPSS18.0.

RESULTS

Demographic and genetic characteristics

The allelic distributions of BDNF (Val/Val = 83, Met/Val = 135, Met/Met = 48) and COMT (Val/Val = 130, Met/Val = 110, Met/Met = 26) were in Hardy–Weinberg equilibrium (BDNF: $\chi^2 = 0.288$, $p = 0.866$; COMT: $\chi^2 = 0.149$, $p = 0.928$). We lumped COMT Met carriers together as in the previous study (Ettinger et al., 2008) because of the quite small number of Met homozygotes. ANOVAs and chi-square tests did not reveal any significant differences between genotype groups with respect to demographic information (age, sex, education) or IQ (all $p > 0.05$) (Table 1).

The rs-FC patterns of the VST

The VST was significantly positively correlated with several brain regions including the medial PFC, orbitofrontal cortex, thalamus, insula, amygdala, hippocampus, parahippocampal gyrus and caudate nucleus ($p < 0.05$, FWE corrected). The VST was also negatively functionally connected to the middle PFC, inferior temporal cortex, lateral parietal cortex and precuneus. The above results were consistent with previous studies (Di Martino et al., 2008; Barnes et al., 2010). A small sensorimotor area was also significantly positively correlated with the VST, but the connectivity was weaker than the other areas. This was supported by

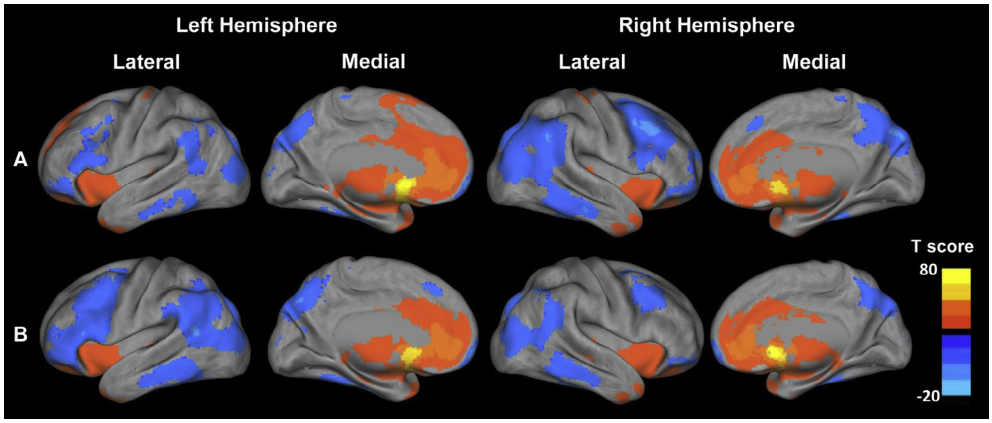

Fig. 1. Resting-state functional connectivity pattern between the bilateral ventral striatum and the rest of the brain across all subjects (FWE corrected $p < 0.05$, clusters > 100). Warm color indicates positive connectivity and cold color indicates negative connectivity. (A) Left ventral striatum connectivity pattern. (B) Right ventral striatum connectivity pattern. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2. Brain regions showing significant functional connectivity with left VST

Volume (mm^3)	Peak voxel (Z)	MNI coordinate			Side	Identified brain region
		x	*y*	*z*		
Left VST: positive correlations						
26,724	inf	−12	16	−6	L	Caudate
	inf	10	16	−4	R	Caudate
	inf	−6	38	0	L	Anterior cingulate
	inf	10	36	−4	R	Anterior cingulate
	inf	−20	30	−12	L	Middle frontal gyrus
	inf	−2	−10	4	L	Thalamus
	inf	−14	46	−18	L	Superior frontal gyrus
	inf	14	48	−18	R	Superior frontal gyrus
	inf	−4	−28	−4	L	Midbrain
	inf	−36	16	−36	L	Superior temporal gyrus
	inf	40	−6	−42	R	Inferior temporal gyrus
	inf	38	12	−38	R	Superior temporal gyrus
	inf	−40	−4	−42	L	Inferior temporal gyrus
	inf	−20	−18	−20	L	Parahippocampal gyrus
	inf	−14	56	14	L	Medial frontal gyrus
	7.69	38	−24	0	R	Insula
	7.60	−42	−24	0	L	Insula
168	inf	24	−38	−4	R	Parahippocampal gyrus
225	6.42	36	−34	70	R	Postcentral gyrus
	5.53	46	−14	62	R	Precentral gyrus
176	6.34	−36	−22	68	L	Precentral gyrus
	5.73	−22	−30	72	L	Postcentral gyrus
	5.18	−30	−30	52	L	Precentral gyrus
Left VST: negative correlations						
2142	inf	−38	−32	−24	L	Parahippocampal gyrus
	7.22	−62	−58	−12	L	Inferior temporal gyrus
	6.27	−32	−48	−16	L	Fusiform gyrus
	5.62	−50	−22	−16	L	Middle temporal gyrus
14,313	inf	42	−30	−26	R	Parahippocampal gyrus
	inf	36	−74	24	R	Middle temporal gyrus
	inf	12	−78	42	L	Precuneus
	inf	56	−50	36	R	Supramarginal gyrus
	inf	44	−60	42	R	Inferior parietal lobule
	inf	−32	−84	18	L	Middle occipital gyrus
	inf	30	−74	40	R	Precuneus
	7.66	48	−52	10	R	Superior temporal gyrus
	7.26	−38	−64	38	L	Inferior parietal lobule
3392	inf	34	66	4	R	Middle frontal gyrus
	inf	−30	54	−16	L	Superior frontal gyrus
	inf	−54	32	12	L	Inferior frontal gyrus
	7.15	−46	52	8	L	Middle frontal gyrus
3937	inf	46	20	40	R	Middle frontal gyrus
	inf	60	16	4	R	Inferior frontal gyrus
341	6.99	−28	6	54	L	Middle frontal gyrus
123	6.29	6	34	42	R	Medial frontal gyrus

previous classic studies that demonstrated that the VST works as a functional interface between emotion regulation and sensorimotor control (Haber et al., 1995; Gopinath et al., 2011). The results for the left and right VST are shown in Fig. 1, Tables 2 and 3.

Interaction effects between BDNF Val66Met and COMT Val158Met in the VST-PFC pathway

We found pronounced interactions of BDNF-by-COMT on the functional connectivity between the bilateral VST-anterior cingulates (ACCs), but no main effects for either the BDNF or the COMT genotype alone based on the ANOVAs ($p > 0.05$, FWE small-volume correction (SVC)). The effects of BDNF Val66Met on the VST-PFC functional connectivity were significantly influenced by COMT (two-way ANOVA, $F(2,258) = 12.49$, $p < 0.05$, FWE SVC). Specifically, subjects with COMT Met alleles revealed an inverted U-shaped between the mean Z scores and increasing numbers of the BDNF Met allele in COMT Met carriers, while individuals with COMT-Val homozygotes displayed a U-shaped (Fig. 2).

DISCUSSION

This is the first study to test the interaction effect of BDNF and COMT on the VST-PFC circuits. The number of BDNF Met alleles was non-linearly related to VST-ACC

Table 3. Brain regions showing significant functional connectivity with right VST

Volume (mm^3)	Peak voxel (Z)	MNI coordinate			Side	Identified brain region
		x	*y*	*z*		
Right VST: positive correlations						
24,654	inf	10	18	−4	R	Caudate
	inf	−10	18	−4	L	Caudate
	inf	−6	38	0	L	Anterior cingulate
	inf	10	38	−6	R	Medial frontal gyrus
	inf	24	26	−12	R	Inferior frontal gyrus
	inf	8	34	10	R	Anterior cingulate
	inf	14	4	4	R	Lentiform nucleus
	inf	4	−10	4	R	Thalamus
	inf	−14	46	−18	L	Superior frontal gyrus
	inf	14	48	−18	R	Superior frontal gyrus
	inf	−2	−26	−2	L	Midbrain
	inf	36	10	−40	R	Superior temporal gyrus
	inf	22	−38	−4	R	Parahippocampal gyrus
	inf	−36	16	−36	L	Superior temporal gyrus
	inf	−18	−18	−18	L	Parahippocampal gyrus
	inf	4	−18	−16	R	Midbrain
Right VST: negative correlations						
2374	inf	42	−30	−26	R	Parahippocampal gyrus
	inf	66	−46	−14	R	Middle temporal gyrus
	5.96	54	−22	−24	R	Middle occipital gyrus
	5.69	50	−12	−26	R	Inferior temporal gyrus
3001	inf	−38	−32	−24	L	Parahippocampal gyrus
	inf	−58	−30	−18	L	Middle temporal gyrus
	7.67	−56	−60	−16	L	Inferior temporal gyrus
6359	inf	−6	68	−12	L	Superior frontal gyrus
	inf	−30	8	50	L	Middle frontal gyrus
	inf	−56	26	8	L	Inferior frontal gyrus
11,878	inf	−50	−54	20	L	Superior temporal gyrus
	inf	−42	−62	44	L	Inferior parietal lobule
	inf	−8	−80	40	L	Precuneus
	inf	14	−74	44	R	Precuneus
	inf	40	−82	18	R	Middle temporal gyrus
	7.53	62	−52	24	R	Supramarginal gyrus
	6.61	48	−5	10	R	Superior temporal gyrus
	6.37	38	−64	42	R	Inferior parietal lobule
1715	inf	44	20	38	R	Middle frontal gyrus
	inf	28	12	54	R	Superior frontal gyrus
121	7.18	34	66	6	R	Middle frontal gyrus
114	6.68	−6	28	46	L	Medial frontal gyrus

Note: inf: infinity; R: right; L: left; VST: ventral striatum. $p < 0.05$, clusters > 100, FWE corrected.

functional connectivity, depending on the COMT genotype. Additionally, functional connectivity of the frontostriatal circuit was a good endophenotype for studying the interactive effects of BDNF and COMT.

It is often the case that a single gene only contributes a small amount to a specific phenotype. Some early studies proposed a negative correlation between the level of prefrontal and striatal DA as well as a role for the PFC modulating the dopaminergic input to the striatum (Bertolino et al., 2000; Jackson et al., 2001). Later studies also found that prefrontal activity has direct or indirect influences on striatal activity, and COMT is involved in the subcortical regulation of phasic DA function (Bilder et al., 2004; Frank and Fossella, 2011). That is, PFC can directly influence the striatal response to dopaminergic signals, or indirectly influences the amount of DA in the VST, which in turn regulates striatal activity. In addition, the BDNF proteins in the VST that primarily originate from the PFC through dopaminergic cortical afferents are essential to the survival of striatal neurons (Baquet et al., 2004) and strongly modulate the activity of the NAc neurons (Guillin et al., 2001). Thus, BDNF, by affecting striatal DA release, may interact with COMT, which has a preferential role in prefrontal DA degradation, through the top-down frontostriatal circuit.

The myriad of brain functions are accomplished by the co-operation between distinct brain regions, which are always involved in a functional circuit or network. rs-FC measures the coherent patterns of low-frequency spontaneous neuronal activity of a circuit. An early study proposed five parallel frontal-subcortical circuits based on anatomical connectivity (Alexander et al., 1986), and a subsequent meta-analysis based on positron emission tomography and fMRI studies also found

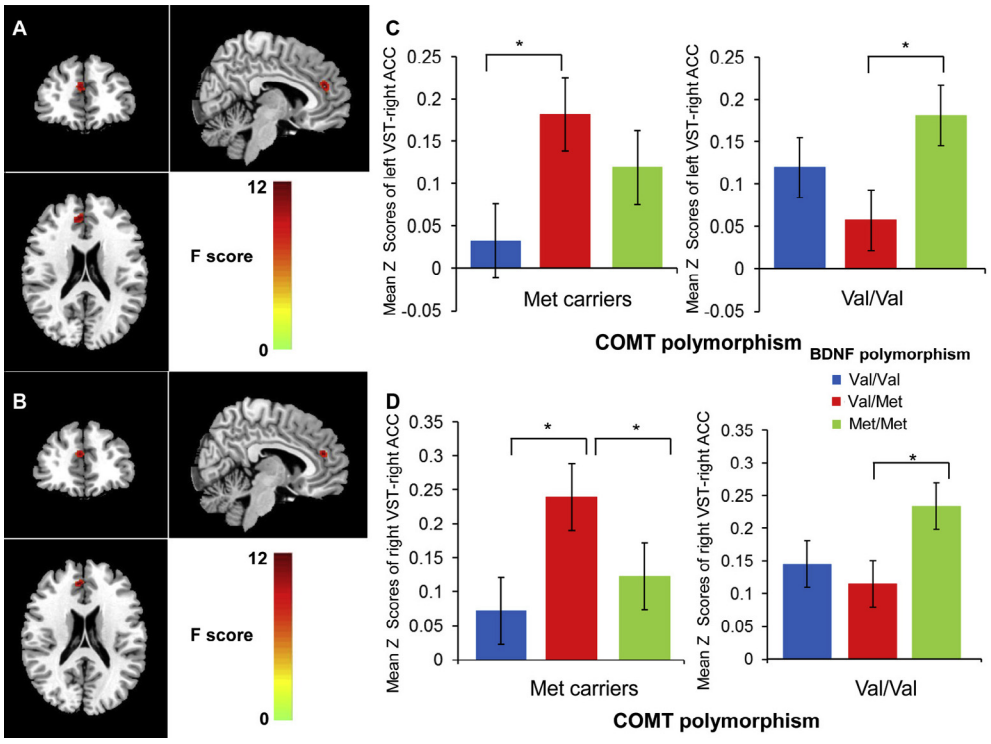

Fig. 2. Interaction of *BDNF Val66Met* and *COMT Val158Met* on VST-PFC connectivity. (A) The significant region shows the *BDNF-COMT* interaction on the functional connectivity between the left ventral striatum and the PFC. (B) The significant region shows the *BDNF-COMT* interaction on the functional connectivity between the right ventral striatum and the PFC (*$p < 0.05$; post hoc *t*-test). (C) The left VST-right ACC connectivity distributions by *BDNF* genotypes in *COMT Met carriers* and *Val homozygotes*. (D) The right VST-right ACC connectivity distributions by *BDNF* genotypes in *COMT Met carriers* and *Val homozygotes* (*$p < 0.05$; post hoc *t*-test).

consistent functional connectivity patterns between the PFC and the striatum (Postuma and Dagher, 2006). These studies firmly suggested that the frontostriatal circuit operates as an integrated whole across widely separated brain regions. In addition, optogenetic research in rodents revealed that optogenetic stimulation of the frontostriatal circuit could recover defective down-regulation of neuron activity in this circuit (Burguiere et al., 2013). Animals, with local lesions of the frontostriatal pathway, have displayed impaired performance in multiple cognition, memory and attention tasks (Annett et al., 1989; Chang et al., 2002; Mair et al., 2002; Jongen-Relo et al., 2003; Christakou et al., 2004). Moreover, the frontostriatal circuit forms a distributed network implicated in processing cognitive information and emotional stimuli. Furthermore, the ACC, part of the midline PFC, acts as a physiological and behavioral signal generator and regulates the top-down frontostriatal system to modulate positive and negative emotions (Watanabe et al., 2015). In addition, anatomical tracing studies performed in rhesus monkeys point out neurons in the ACC project to the ventral striatum (Selemon and Goldman-Rakic, 1985). Emerging evidences revealed that DA levels, gray matter volume for ACC and rs-FC strength with ACC in the right hemisphere were greater than the left (Afonso et al., 1993; Paus et al., 1996; Huster et al., 2007; Yan et al., 2009; Watanabe et al., 2015). Hence, functional connectivity with the right ACC might be sensitive to slight changes in DA levels, which our research indicates are regulated by the interaction of the BDNF and COMT genes.

In general, epistasis, defined as the interaction between genes on complex traits, is likely to regulate the frontostriatal functional circuit (Cordell, 2002; Marchini et al., 2005). Although originally epistasis referred to the effect of a gene or a single locus that blocks the action of another gene or locus, it has now come to refer to the much broader scope of interactions between genes at different loci. Prior investigations testing the effects of BDNF Val66Met on brain structure and function have reported contradictory results. Most studies assumed that BDNF Val66Met works in a Met dosage-dependent manner, that is, according to the dosage effect of the Met allele on the activity-dependent secretion of BDNF (Chen et al., 2006) or according to the levels of n-acetyl-aspartate (NAA) in the hippocampus (Egan et al., 2003). Indeed, our previous study demonstrated a Met-dose effect on the surface area of the anterior insula and its related functional network (Wang et al., 2014). However, some studies have indicated a nonlinear effect of BDNF (Kleim et al., 2006; Forde et al., 2013). The inconsistency across these studies may have been caused by differences in sample size, methodology and racial diversity between samples. However, this inconsistency might also be attributable to ignoring the potential for gene–environment or gene–gene interactions. Our present results revealed an epistatic interaction between BDNF and COMT on VST-PFC connectivity; that is, a

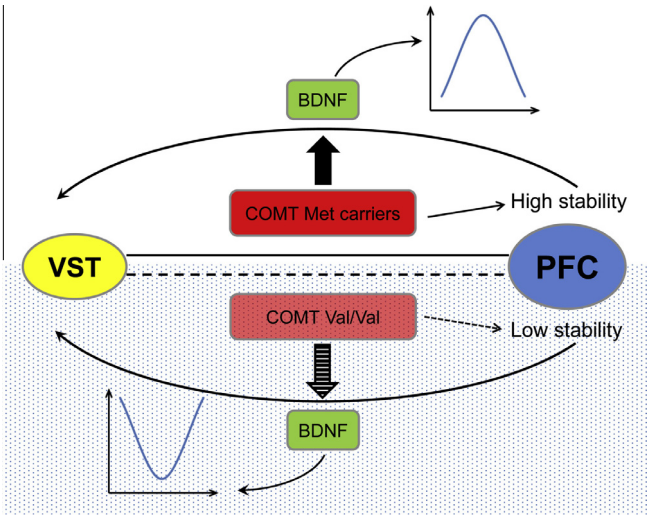

Fig. 3. Sketch map of the epistatic interaction of *BDNF Val66Met* and *COMT Val158Met*. The gray area indicates that the *COMT Val allele* results in low stability in the frontostriatal circuit. The lack of DA in the PFC in turn causes a loss of control of the impact of *BDNF* on the ventral striatum. The curved line at the bottom-left indicates that *BDNF Val66Met* impacted the VST-PFC functional connectivity in a U-shaped in *COMT Val/Val*. The white area indicates that the *COMT Met allele* results in a high stability of the frontostriatal circuit. The effect of *BDNF* on dopamine release and activity of the ventral striatum are brought under good control by the *COMT Met allele* by regulating the connections of the VST-PFC. The curved line at the upper-right indicates that *BDNF Val66Met* impacted VST-PFC functional connectivity in an inverted U-shaped in *COMT Met carriers*.

nonlinear effect of COMT on brain function seems to alter the dosage effect of BDNF Val66Met.

Interestingly, we observed a completely opposite effect of BDNF Val158Met in COMT Met carriers and Val/Val homozygotes. BDNF has been reported to impact dopaminergic activity and mediate dopaminergic neurons (Poo, 2001; Narita et al., 2003), and COMT Val158Met modulates PFC-dependent functions in an inverted U-shaped manner (Nagel et al., 2008). Theoretically, COMT Met carriers have relatively elevated DA levels and a high stability of the frontostriatal circuit (Savitz et al., 2006). Under the epistatic interaction of BDNF and COMT, the higher stability of the frontostriatal circuit in COMT Met carriers may generate an optimally functioning state of the dopaminergic neurons in the PFC, which in turn monitors the effect of BDNF on DA release and activity of the VST by regulating the connections of the VST-PFC (Fig. 3). However, COMT Val/Val carriers have lower DA levels and lower stability of the frontostriatal circuit, factors which are unfavorable for the functioning of the frontostriatal circuit (Winterer and Weinberger, 2004). The lack of DA in the PFC may cause a loss of control of the impact of BDNF on the VST. Based on the assumption of a Met-dose effect of BDNF in COMT Val homozygotes, BDNF Val/Met carriers seem to have had reduced coupling between VST and PFC compared with the BDNF Val homozygotes, but not enough to reach statistical significance. In addition, under the highest risk state of BDNF, that is, Met–Met, and low DA levels in the PFC, the frontostriatal circuit exhibits the lowest dopaminergic activity, but this can be compensated for by strengthening the connectivity in the frontostriatal circuit.

CONCLUSION

Our findings seem to offer hypotheses that can lead to an improved understanding of the interaction between BDNF and COMT on the function of brain regions or networks involved in the DA pathway. Future studies could focus on identifying novel dopaminergic genes and exploring the whole DA system. Studying the entire system may help us to clarify the biological mechanisms underlying individual differences in behavior performance, as manifested by dopaminergic neurons or modulated by DA levels.

CONTRIBUTIONS

T.J. supervised the study. C.W. and B.L. performed the experimental works, analyzed results, and wrote the manuscript with assistance from H.L., L.F., J.L., X.Z., C.Q., C.Y. All authors discussed the results.

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