



The cortical surface area of the insula mediates the effect of *DBH* rs7040170 on novelty seeking



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ABSTRACT

Novelty seeking (NS) is a personality trait important for adaptive functioning, but an excessive level of NS has been linked to psychiatric disorders such as ADHD and substance abuse. Previous research has investigated separately the neural and genetic bases of the NS trait, but results were mixed and neural and genetic bases have yet to be examined within the same study. In this study, we examined the interrelationships among the dopamine beta-hydroxylase (*DBH*) gene, brain structure, and the NS trait in 359 healthy Han Chinese subjects. We focused on the *DBH* gene because it encodes a key enzyme for dopamine metabolism, NS is believed to be related to the dopaminergic system and has been reported associated with *DBH* variation. Results showed a significant positive association between the cortical surface area of the left insula and NS score. Furthermore, the *DBH* genetic polymorphism at the SNP rs7040170 was strongly associated with both the surface area of the left insula and NS score, with G carriers having a larger left insula surface area and a higher NS score than AA homozygotes. Subsequent path analysis suggested that the insula partially mediated the association between the *DBH* gene and the NS trait. Our data provided the first evidence for the involvement of the insula in the dopamine–NS relationship. Future studies of molecular mechanisms underlying the NS personality trait and related psychiatric disorders should consider the mediation effect of the neural structure.

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Introduction

Novelty seeking (NS) is a personality trait characterized by a preference for exploratory and novel activities, avoidance of monotony and routine, and extravagance in approach to reward cues (Cloninger, 1987; Cloninger et al., 1993). This trait contributes to adaptive functioning. High NS individuals are impulsive, excitable, and quick-tempered, while low NS individuals are rigid, stoic, and slow-tempered (Cloninger, 1986; Cloninger et al., 1993). However, excessive NS has been linked to psychiatric disorders (Richter and Brandstrom, 2009), such as ADHD (Instanes et al., 2013; Jacob et al., 2014), pathological gambling (Kim and Grant, 2001) and substance abuse (Milivojevic et al., 2012), while reduced levels of NS are correlated with obsessive–compulsive disorders (Lyyo et al., 2001). A number of genetic and neuroimaging studies have attempted to examine the biological basis of NS, but results have been

mixed and the combined genetic–neural mechanism of the NS remains to be explored.

Following Cloninger's model (Cloninger, 1986), much of the genetic work of the NS trait has focused on candidate genes affecting the dopamine (DA) system, and their genetic effects on NS (Davila et al., 2013; Montag et al., 2010; Munafò et al., 2008). In this study we focused on the *DBH* gene, located on chromosome 9q34. It encodes dopamine β-hydroxylase (DβH) protein (Kaufman and Friedman, 1965) and is a major quantitative trait locus of plasma DβH activity (Zabetian et al., 2001). DβH protein catalyzes the conversion of DA to norepinephrine (NE) (Levin et al., 1960) in synaptic vesicles and thereby influences extracellular DA level. Hence, one might speculate that variation in the *DBH* gene could impact NS by virtue of its effect on dopamine levels. This hypothesis has been supported by recent studies linking the *DBH* gene to NS (Hess et al., 2009) and several psychiatric disorders related to excessive NS, such as ADHD (Carpentier et al., 2013) and addiction (Preuss et al., 2013). However, the mechanism for these gene–behavior associations, especially neural structure as mediating factors (or neural endophenotypes (Goldberg and Weinberger, 2004; Meyer-Lindenberg and Weinberger, 2006)), remains unclear. Therefore, this study aimed to study the role of *DBH* gene on neural structure underlying NS. Our

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results should help to better elucidate the influence of the *DBH* gene on NS as well as related neuropsychiatric disorders.

Neuroimaging studies have examined the correlation between NS and cortical volume in several brain regions (Gardini et al., 2009; Iidaka et al., 2006; Schilling et al., 2013b; Van Schuerbeek et al., 2011), however, these findings are mixed. Some research found that NS was associated with cortical volume in the middle frontal gyrus (Iidaka et al., 2006) and orbitofrontal cortex (Schilling et al., 2013b), whereas some others found associations with regions such as the posterior cingulate regions (Gardini et al., 2009; Van Schuerbeek et al., 2011) and cerebellum (Van Schuerbeek et al., 2011). These inconsistent results might be attributed to differences in sample size, methods, or statistical model specifications (Hu et al., 2011). Another possible issue is that most studies have examined cortical volume as a whole, while ignoring the two individual components that comprise it, cortical thickness and surface area. Cortical volume is the product of cortical thickness and surface area, which are genetically (Panizzon et al., 2009) and phenotypically (Winkler et al., 2010) independent. Moreover, these two measures are distinct aspects of the neural architecture and have different developmental trajectories (Lyall et al., 2014). Thus far, to our knowledge, the majority of structural neuroimaging studies of the NS trait have investigated the volume measure as a whole (Gardini et al., 2009; Iidaka et al., 2006), except for one study which estimated the cortical thickness measure but only focused on a facet of the NS trait (impulsiveness) (Schilling et al., 2013a). Given this gap in the literature, the respective contributions of surface area and cortical thickness to the NS trait should be investigated. This could not only help us to clarify the neural structure basis of the NS trait, but could also provide appropriate endophenotypes for imaging genetics studies (Winkler et al., 2010), to better understand the genetic–neural basis of the NS trait.

The aim of the present study was to examine the brain structure underpinnings of NS trait and to understand the gene–brain–behavior relationships among the *DBH* gene, brain structure, and the NS score. Therefore, we first evaluated the respective effects of cortical surface area and cortical thickness on the NS trait in a large healthy Han Chinese sample. Then we tested the association between the single nucleotide polymorphisms (SNPs) in and nearby the *DBH* gene and NS-related brain structures. Finally, to clarify the role of the brain in the complex interrelations, we performed path analyses to test whether brain structure served as a mediator between the *DBH* gene and the NS trait. We hypothesized that variations in the *DBH* gene would be related to cortical structure and NS score, and furthermore, that cortical structure would mediate the association between the genetic variations and NS score.

Material and methods

Subjects

Three-hundred and fifty nine healthy Han Chinese subjects (173 females, mean age = 19.4 ± 1.1 years) participated in the study. All subjects had no history of neurologic or psychiatric disorders, and were not taking any medications that could interfere with their ability to complete a questionnaire or provide structural MRI data. This study was approved by the Ethics Committee of School of Life Science and Technology at the University of Electronic Science and Technology of China. All participants gave informed written consent.

Genotyping

After blood sample collection, genomic DNA was extracted using the E.Z.N.A.TM Blood DNA Kit (Omega Bio-Tek, Georgia, US). All samples were genotyped using the standard Illumina genotyping protocol (Illumina, Inc). As described in Supplemental Materials Table S1, 51 SNPs located in or within 100 kb around the *DBH* gene on chromosome 9 were selected. These SNPs covered most of the linkage disequilibrium

(LD) blocks in this region, as defined for the Chinese sample included in the HapMap Project (<http://www.hapmap.org/phase3/>). Five of the 51 SNPs failed to pass minor allele frequency (MAF) > 0.05 and were excluded for further analyses. The remaining 46 SNPs met the criteria for Hardy–Weinberg equilibrium (HWE) $p > 0.01$ and genotype call rate > 0.98 and were included in subsequent analyses. When fewer than five participants were categorized as either heterozygotes or minor allele homozygotes for a SNP, the two genotype groups were combined in further analysis.

Novelty seeking measure

Each subject was asked to complete the Chinese version of the Temperament and Character Inventory-Revised (TCI-R) (Chen et al., 2011; Cloninger, 1994). This inventory was translated from English to Chinese and back translated and verified through a bilingual group discussion, and the resulting Chinese versions had high internal consistency (Chen et al., 2011). The NS subscale of the TCI-R measures individual differences in the extent to which a person is quick tempered, impulsive, extravagant, and disorderly versus rigid, stoical, and orderly (Cloninger et al., 1993). The total score on the NS subscale was used in the current study.

Image acquisition and preprocessing

MRI scans were performed with an MR750 3.0 Tesla magnetic resonance scanner (GE Healthcare). High-resolution 3D T1-weighted brain volume (BRAVO) MRI sequence was performed with the following parameters: TR = 8.16 ms, TE = 3.18 ms, flip angle = 7°, FOV = 256 mm × 256 mm, voxel size = $1 \times 1 \times 1$ mm³, and 188 slices. All the raw MRI data were inspected by two experienced radiologists who were blind to genotype information.

MRI data were analyzed with atlas-based FreeSurfer software (<http://surfer.nmr.mgh.harvard.edu>, version 5.3.0). The cortical surface was constructed through an automated procedure, involving segmentation of the white matter, classification of the gray/white matter boundary, inflation of the folded surface, and automatic correction of topological defects (Dale et al., 1999; Fischl and Dale, 2000). After the initial surface model had been constructed, measures of cortical surface area were calculated by computing the area of each triangle of a standardized tessellation. Then all of the individual reconstructed cortical surfaces were aligned to an average template with a surface-based registration algorithm. Quality control of scan images and segmentation was assured by visual inspection of the whole cortex of each subject and manual editing following the standard editing rules. Any inaccuracies in Talairach-transformation, skull stripping, and segmentation were also manually corrected, and re-inspected. A high correlation between these automatic measures and manual measures in vivo and ex vivo has been demonstrated (Desikan et al., 2006). Cortical thickness and surface area maps were then smoothed using a Gaussian kernel (20 mm FWHM).

Statistical analysis

After surface reconstruction, vertex-by-vertex analyses of cortical thickness and surface area were performed separately, by using a general linear model to estimate the association between the morphological measure at each vertex and the NS score. Gender and age were included as covariates to avoid potential confounding effects (Smith et al., 2007). Significance maps were then corrected for multiple comparisons with cluster-based Monte Carlo simulations with 5000 permutations (using the FreeSurfer program `mri_glmfit-sim`, corrected for two spaces). Finally, because thickness analysis did not yield any significant results, the region significantly correlated with the NS score (corrected $p < 0.05$) in the cortical surface area analysis, the left insula, was extracted. We mapped this region onto the average reconstructed surface for visual display,

and calculated the mean surface area for each subject for subsequent analysis.

Linear regression models were then used to detect the associations between each SNP and the mean surface area of the left insula. Additive genetic models (i.e., additive effects of allele dosage) were used, with sex and age as covariates. Statistical significance level was set at $p < 1.09 \times 10^{-3}$ (0.05/46 [SNPs], i.e., Bonferroni correction). Only one SNP (rs7040170) passed the significance level and was selected for further analysis. Allelic association tests were carried out by using plink v1.07 (Purcell et al., 2007). The genotype frequencies (AA = 304, AG = 53, GG = 2) were within the Hardy–Weinberg equilibrium ($\chi^2 = 0.036$, $p = 0.850$). Given that there were only two GG homozygotes, we combined the two subjects with the AG group and comparisons were carried out between AA homozygotes and G carriers.

Mediation model

A mediation model was set up, with the surface area of the left insula as a mediator, to test the direct and indirect effects of *DBH* rs7040170 on NS score. We used the PROCESS macro developed by Hayes (2012) to test for a mediation model (model 4), with NS score as the outcome variable, the *DBH* rs7040170 polymorphism as the predictor, and the average surface area of the left insula as the mediator, with sex and age as covariates. The model estimates the total, direct (path from *DBH* genotype to NS score), and indirect (path from *DBH* genotype to NS score through the left insula) relations, to clarify whether the effect of *DBH* on NS was significantly mediated through the surface area of the left insula. Statistical significance for the mediators was established by bootstrapped 95% confidence intervals (CI) with 5000 iterations.

Results

The participants' NS scores ($M = 101.2$, $SD = 11.3$) were comparable to those of another Chinese sample in a previous study that used the same inventory (Lei et al., 2014).

Then the cortical surface maps for each individual was calculated, and regression analyses with NS scores were conducted. Significant positive correlations between the surface area in the left insular cortex (including a few parts of the superior temporal gyrus) and NS score were observed (corrected cluster-wise $p = 0.009$, Monte-Carlo correction, see Fig. 1A), whereas no significant correlation was found between cortical thickness and NS score.

Then we conducted a series of association tests using Plink, to test the association between the polymorphism at each *DBH* SNP and the mean surface area of the left insula. As presented in Fig. 1B and Supplemental Materials Table S2, only one SNP (rs7040170) located about 62 kb downstream of the *DBH* gene showed a significant association with the left insula surface area after Bonferroni correction ($t = 3.48$, uncorrected $p = 5.7 \times 10^{-4}$), after controlling for the potential confounding effects of sex and age. Further regression analyses also showed a significant correlation between this SNP and the NS score ($t = 2.63$, $p = 9.0 \times 10^{-3}$, again with sex and age as covariates). For rs7040170, G carriers showed both larger left insula surface area and higher NS score than AA homozygotes (see Table 1 and Fig. 1C, D).

Given the three concurrent relationships between the gene, neural structure and behavior, a path model was applied to examine whether the insula mediated the effect of *DBH* rs7040170 on NS score. Analysis in PROCESS revealed significant direct paths including a path from *DBH* rs7040170 genotype to NS score ($B = 0.31$, $SE = 0.15$, $p = 0.04$), a path from *DBH* rs7040170 genotype to insula surface area ($B = 0.45$, $SE = 0.13$, $p = 0.0006$) and a path from the insula surface area to NS

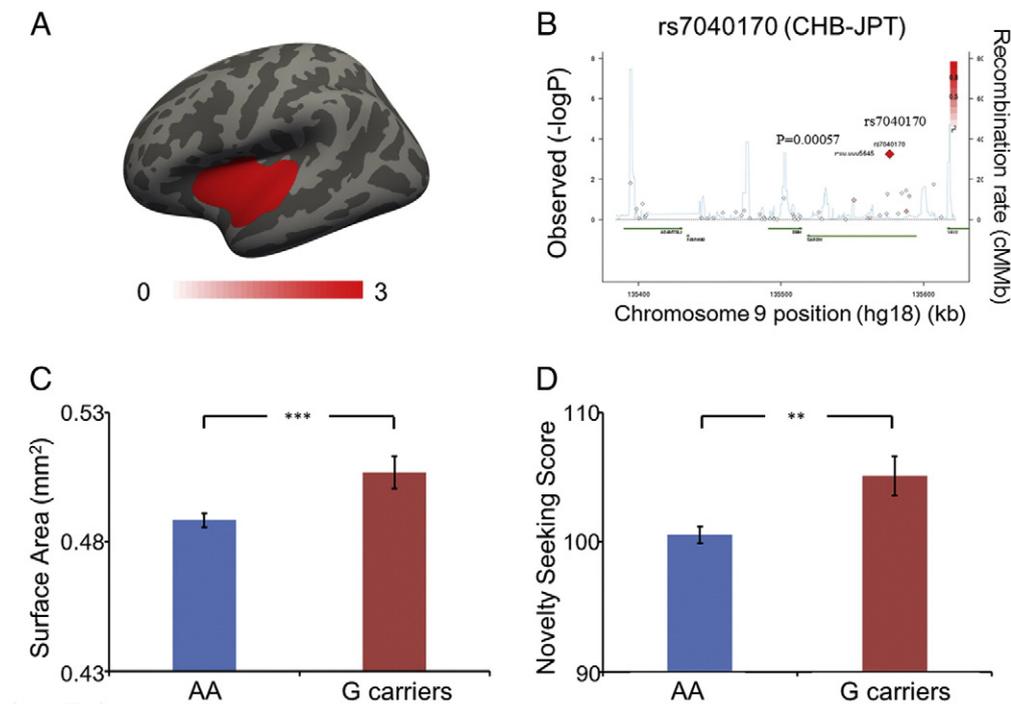


Fig. 1. Associations among the *DBH* gene, cortical surface area, and novelty seeking score. A, The region in red showed a significant linear increase in cortical surface area with increasing novelty seeking score, $p < 0.05$, Monte-Carlo corrected for two hemispheres. B, Associations between 46 SNPs in or within 100 kb around the *DBH* gene region and surface area of the survived region with sex and age as covariates. All SNPs were plotted with their p values against their genomic position, with the most significant SNP in the region indicated as a diamond and other SNPs shaded according to their pair-wise correlation (r^2) with the signal SNP. The light blue line represented the estimated recombination rates. Gene annotations were shown as dark green lines. The regional plots were generated using the SNAP program (Johnson et al., 2008). C and D, Surface area (C) and novelty seeking score (D) in the survived region (means and standard errors) in rs7040170 AA homozygotes and AG heterozygotes. ** $p < 0.01$ (ANOVA). *** $p < 0.001$ (ANOVA).

Table 1
Subject demographics by rs7040170 genotype.

DBH	Mean (SD)		p value
	AA (N = 304)	G carriers (N = 55)	
Genotype			
Male/female	160/144	26/29	0.468 ^a
Age (years)	19.37 (1.07)	19.55 (1.20)	0.270 ^b
Average surface area of each vertex in insula (mm ²)	0.49 (0.05)	0.51 (0.05)	5.662E-4 ^c
Novelty seeking score	100.56 (11.08)	105.11 (11.84)	0.009 ^c

^a p value (Pearson χ^2 test).
^b p value (ANOVA).
^c p value (main effects of rs7040170 genotype, ANCOVA with gender and age as covariates).

score ($B = 0.16$, $SE = 0.06$, $p = 0.0058$) (model depicted in Fig. 2). There was also a significant indirect path from DBH rs7040170 genotype to NS score through insula surface area ($\alpha\beta = 0.07$, $SE = 0.03$, $p = 0.03$, $95\% CI = [0.02, 0.17]$). This suggested that the effect of DBH rs7040170 genotype on the NS trait was partially accounted for by genotype-related variations in the insula's surface area.

Discussion

The present study found significant associations between the DBH rs7040170, surface area of the left insula, and NS scores. Furthermore, path analyses indicated that neural structure (i.e., surface area of the left insula) mediated the effect of the DBH rs7040170 polymorphisms on the NS trait. These results together provided possible genetic and neural mechanisms underlying the NS trait and psychiatric disorders related to abnormal NS scores, such as ADHD, pathological gambling and substance abuse (Instanes et al., 2013; Jacob et al., 2014; Kim and Grant, 2001; Milivojevic et al., 2012).

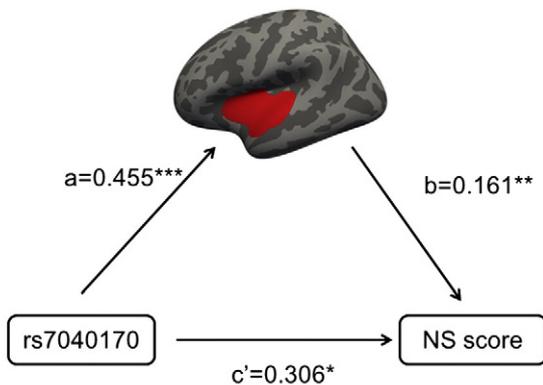
One of the major findings of this study was the correlation of NS score with cortical surface area but not with cortical thickness. This result confirmed our conjecture that these two measurements should be considered separately in studies of NS trait. According to the radial unit hypothesis (Rakic, 1988), these two phenotypes have distinct

neurodevelopmental mechanisms, as cortical surface area is influenced by the number of columns, whereas cortical thickness is influenced by the number of cells within a column. The significant contribution of surface area in our study might be supported by one or more of the following. First, individual differences in cortical gray matter volume were found to be more closely related to differences in cortical surface area than cortical thickness (Winkler et al., 2010). In addition, cortical surface area became dramatically larger during evolution while cortical thickness remained relatively conserved (Rakic, 2009). Last but not least, reduced surface area has been found to be linked to psychiatric disorders (Rimol et al., 2012), and has been proven more useful than cortical thickness in elucidating the associations between brain structure and cognitive endophenotypes of psychiatric disorders (Vuoksimaa et al., 2014).

The positive association between the left insular surface area and NS scores in our study is consistent with previous research implicating the insula in impulsiveness (Lee et al., 2008) and risk and reward processing (Hauser et al., 2015; Mohr et al., 2010). Our results are also in line with previously documented positive correlations between regional cerebral blood flow (rCBF) in the insula and NS (Sugiura et al., 2000), and insular surface area and impulsivity (Kaag et al., 2014). Besides, this anatomical association is consistent with a recent study on patients suffering substance abuse who were found to score high on NS (Bell et al., 2014) and showed increased surface area in the insular cortex (Kaag et al., 2014). Previous research has indicated that lesions to the insula to be associated with a reduction in addictive behaviors (Naqvi et al., 2007). In that case, it is clear that the anatomy of the insula may constrain its function. Hence, we speculate that a small regional surface area of insular cortex could lead to decreased tendency of impulsivity and to risky behaviors through the insula's role in the processing of interoceptive cues as conscious feeling, such as urge (Naqvi and Bechara, 2009), thus associated with a lower NS score. Thus, we believe that the left insular surface area represents a promising biomarker for delineating the brain structural substrate for human NS trait and for understanding the neurobiological correlates for NS-related psychiatric conditions, such as ADHD (Jacob et al., 2014) and substance abuse (Bell et al., 2014).

Our study also found a lateralized association between the left insular structure and the NS trait. This finding is consistent with earlier studies that documented positive correlations between NS and rCBF in the left but not the right insula (Sugiura et al., 2000) and cigarette smoking induced cortical dopamine release in the left but not the right insula (Wing et al., 2015). This lateralization could be due to hypothesized differences in functionality of the left and right sides of the forebrain (Craig, 2005). Specifically, the left forebrain is predominantly associated with parasympathetic activity, which may underlie positive affect and approach behaviors, whereas the right forebrain is thought to be associated with sympathetic activity, which may underlie negative affect and avoidance behaviors. This left-lateralized result also concurs with previous EEG (Sutton and Davidson, 1997), fMRI (Berkman and Lieberman, 2010) and repetitive transcranial magnetic stimulation studies [rTMS, (van Honk and Schutter, 2006)] and reviews (Harmon-Jones et al., 2010), all of which demonstrated the lateralization of approach tendencies to the left hemisphere. Moreover, such leftward lateralization has also been reported in psychiatric disorders related to excessive NS, such as ADHD (Bedard et al., 2014) and substance abuse (Krpotic et al., 2013).

We also found that the rs7040170 G allele was associated with a larger insular surface area. There are two possible explanations for this association. One came from the influence of rs7040170 variation on D β H activity. Rs7040170 is localized in the SARDH gene, which is adjacent to, bounded with, and inhabits the same topological domain with the DBH gene (Dixon et al., 2012). In a recent genome-wide association study (GWAS), rs7040170 was identified as one of the SNPs significantly associated with plasma D β H activity ($p = 1.31 \times 10^{-14}$) (Mustapic et al., 2014). Specifically, the G allele is associated with higher



Direct effect of rs7040170 on NS score = 0.306, 95%CI = [0.02, 0.59]; Indirect effect = 0.073, 95%CI = [0.02, 0.17]

Covariates: Gender, Age

Bootstrap 5000 times

Fig. 2. rs7040170-insula-NS score mediation model after controlling for gender and age. Path coefficients in the graph were standardized regression weights. CI, confidence interval. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

plasma D β H activity and correlated with higher D β H activity in cerebrospinal fluid (CSF) (O'Connor et al., 1994). D β H is expressed early in embryonic development (Yew et al., 1995) and increases in late gestation, and may influence the cortical surface area through its roles in cell proliferation, migration and differentiation (Tiu et al., 2003). Another possibility is that rs7040170 G allele may be associated with larger insula surface area through its influence on lower DA level, and then a negative effect of dopamine on insula. The hypothesized effect is supported as the rs7040170 G allele related to higher D β H activity could lead to lower dopamine levels, since D β H catalyzes the conversion of dopamine to noradrenaline (Levin et al., 1960). Moreover, experimental studies have found that the administration of DA receptor agonists decreased rCBF in the insula (Black et al., 2002) and that DAergic depletion increased insular activity (da Silva Alves et al., 2011). The rs7040170–insula relationship can also be inferred as the human insula receives relatively rich DA innervation (Gaspar et al., 1989), expresses DA receptors (Hurd et al., 2001), and is strongly affected by variation in DAergic neurotransmission. These findings together support the influence of the *DBH* rs7040170 polymorphism on insular surface area.

Additionally, a positive correlation was found between the G allele of *DBH* rs7040170 and NS score. A possible explanation for this finding is that the G allele may lead to higher D β H activity and reduced dopamine level, which could in turn lead to higher NS. Several studies support this hypothesis. First, as previously mentioned, the G-allele of rs7040170 has been found to be positively associated with D β H activity (Mustapic et al., 2014). Second, studies suggest that D β H function is positively associated with NS-related behaviors. For instance, higher plasma D β H activity has been found to be related to extraversion (Roy and Brockington, 1987), a trait that is positively correlated with NS (Kristensen et al., 2009), and D β H inhibitors have been found to suppress addictive behaviors (Schroeder et al., 2010). These aforementioned associations are likely due to the negative effects of D β H on dopamine levels (Levin et al., 1960). According to Cloninger's theory (1986), lower basal dopaminergic activity may be associated with more intense orienting responses to novel stimuli, thus resulting in higher NS. These studies together indicated that D β H function may underlie NS-related behaviors through its role on DA level.

In the subsequent path analysis, the insula mediated the effect of *DBH* rs7040170 on NS scores. This result echoed the widely accepted hypothesis in gene–brain–behavior research (Goldberg and Weinberger, 2004; Green et al., 2008) and existing studies (Buckholtz et al., 2008; Fakra et al., 2009; Green et al., 2013) that genetic effects on behaviors are mediated by neural substrates. Many studies have found that the insular cortex regulates dopaminergic activity associated with the NS trait. For instance, a negative correlation between insular cortex DA receptor binding and the NS score has been demonstrated (Suhara et al., 2001). A later study also reported an inverse correlation between DA receptor availability in the insula and NS (Kaasinen et al., 2004). In short, the observed mediation effect was consistent with the reported rich DA innervation in the insula (Gaspar et al., 1989) and further supported Cloninger's model regarding associations between the NS trait and central dopaminergic pathways (Cloninger, 1986).

Further research is needed to support our findings. First, although associations between *DBH* rs7040170, the insula, and NS score were identified in the present research, these correlational results are phenomenological and do not imply causation. For instance, brain–behavior associations could reflect either a biological predetermination or experience-driven plasticity (Hyde et al., 2009). More systematic studies using animal models and other techniques are required to explain these relationships. Second, our sample was comprised of only one ethnic group (i.e., Han Chinese) and only young adults. While this homogeneity is typically considered as an advantage for genetic studies (i.e., in helping with avoidance of ethnic stratification and other confounding effects), it also limits the generalizability of our results to other populations. It has been demonstrated that the relationships between gene, brain and behavior can be modulated by ethnicity

(Long et al., 2013), age (Richter-Schmidinger et al., 2011), and gender (Kazantseva et al., 2014). Also, rs7040170 has different minor allele frequencies (MAF) in different ethnic populations based on the HapMap Data (www.hapmap.org, see Table S1). Therefore, to obtain confirmatory evidence, this association should be explored in future studies in samples with different age groups and ethnicity.

Conclusions

In conclusion, this study provided the first evidence of interrelationships between the *DBH* gene, insula morphology, and the NS trait, and the mediating effect of the brain between the gene and behavior in a sample of healthy Chinese individuals. Our findings suggest that cortical surface area may be a promising endophenotype in imaging genetic studies, and that future studies of gene–personality associations should consider the brain mediation effect.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.neuroimage.2015.05.033>.

References

- Bedard, A.C., Newcorn, J.H., Clerkin, S.M., Krone, B., Fan, J., Halperin, J.M., Schulz, K.P., 2014. Reduced prefrontal efficiency for visuospatial working memory in attention-deficit/hyperactivity disorder. *J. Am. Acad. Child Adolesc. Psychiatry* 53 (1020–1030), e1026.
- Bell, R.P., Foxe, J.J., Ross, L.A., Garavan, H., 2014. Intact inhibitory control processes in abstinent drug abusers (I): a functional neuroimaging study in former cocaine addicts. *Neuropharmacology* 82, 143–150.
- Berkman, E.T., Lieberman, M.D., 2010. Approaching the bad and avoiding the good: lateral prefrontal cortical asymmetry distinguishes between action and valence. *J. Cogn. Neurosci.* 22, 1970–1979.
- Black, K.J., Hershey, T., Koller, J.M., Videen, T.O., Mintun, M.A., Price, J.L., Perlmutter, J.S., 2002. A possible substrate for dopamine-related changes in mood and behavior: prefrontal and limbic effects of a D3-preferring dopamine agonist. *Proc. Natl. Acad. Sci. U. S. A.* 99, 17113–17118.
- Buckholtz, J.W., Callicott, J.H., Kolachana, B., Hariri, A.R., Goldberg, T.E., Genderson, M., Egan, M.F., Mattay, V.S., Weinberger, D.R., Meyer-Lindenberg, A., 2008. Genetic variation in MAOA modulates ventromedial prefrontal circuitry mediating individual differences in human personality. *Mol. Psychiatry* 13, 313–324.
- Carpentier, P.J., Arias Vasquez, A., Hoogman, M., Onnink, M., Kan, C.C., Kooij, J.J., Makkinje, R., Iskandar, S., Kiemeneys, L.A., de Jong, C.A., Franke, B., Buitelaar, J.K., 2013. Shared and unique genetic contributions to attention deficit/hyperactivity disorder and substance use disorders: a pilot study of six candidate genes. *Eur. Neuropsychopharmacol.* 23, 448–457.
- Chen, C., Moyzis, R., Dong, Q., He, Q., Zhu, B., Li, J., Li, H., Lessard, J., 2011. Sex modulates the associations between the COMT gene and personality traits. *Neuropsychopharmacology* 36, 1593–1598.
- Cloninger, C.R., 1986. A unified biosocial theory of personality and its role in the development of anxiety states. *Psychiatr. Dev.* 4, 167–226.
- Cloninger, C.R., 1987. A systematic method for clinical description and classification of personality variants. A proposal. *Arch. Gen. Psychiatry* 44, 573–588.
- Cloninger, C.R., 1994. Temperament and personality. *Curr. Opin. Neurobiol.* 4, 266–273.
- Cloninger, C.R., Svrakic, D.M., Przybeck, T.R., 1993. A psychobiological model of temperament and character. *Arch. Gen. Psychiatry* 50, 975–990.

- Craig, A.D., 2005. Forebrain emotional asymmetry: a neuroanatomical basis? *Trends Cogn. Sci.* 9, 566–571.
- da Silva Alves, F., Schmitz, N., Fígee, M., Abeling, N., Hasler, G., van der Meer, J., Nederveen, A., de Haan, L., Linszen, D., van Amelsvoort, T., 2011. Dopaminergic modulation of the human reward system: a placebo-controlled dopamine depletion fMRI study. *J. Psychopharmacol.* 25, 538–549.
- Dale, A.M., Fischl, B., Sereno, M.I., 1999. Cortical surface-based analysis. I. Segmentation and surface reconstruction. *Neuroimage* 9, 179–194.
- Davila, W., Basterreche, N., Arrue, A., Zamalloa, M.I., Gordo, E., Davila, R., Gonzalez-Torres, M.A., Zumarraga, M., 2013. The influence of the Val158Met catechol-O-methyltransferase polymorphism on the personality traits of bipolar patients. *PLoS One* 8, e62900.
- Desikan, R.S., Segonne, F., Fischl, B., Quinn, B.T., Dickerson, B.C., Blacker, D., Buckner, R.L., Dale, A.M., Maguire, R.P., Hyman, B.T., Albert, M.S., Killiany, R.J., 2006. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage* 31, 968–980.
- Dixon, J.R., Selvaraj, S., Yue, F., Kim, A., Li, Y., Shen, Y., Hu, M., Liu, J.S., Ren, B., 2012. Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature* 485, 376–380.
- Fakra, E., Hyde, L.W., Gorka, A., Fisher, P.M., Munoz, K.E., Kimak, M., Halder, I., Ferrell, R.E., Manuck, S.B., Hariri, A.R., 2009. Effects of HTR1A C(-1019)G on amygdala reactivity and trait anxiety. *Arch. Gen. Psychiatry* 66, 33–40.
- Fischl, B., Dale, A.M., 2000. Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc. Natl. Acad. Sci. U. S. A.* 97, 11050–11055.
- Gardini, S., Cloninger, C.R., Venneri, A., 2009. Individual differences in personality traits reflect structural variance in specific brain regions. *Brain Res. Bull.* 79, 265–270.
- Gaspar, P., Berger, B., Febvret, A., Vigny, A., Henry, J.P., 1989. Catecholamine innervation of the human cerebral cortex as revealed by comparative immunohistochemistry of tyrosine hydroxylase and dopamine-beta-hydroxylase. *J. Comp. Neurol.* 279, 249–271.
- Goldberg, T.E., Weinberger, D.R., 2004. Genes and the parsing of cognitive processes. *Trends Cogn. Sci.* 8, 325–335.
- Green, A.E., Munafo, M.R., DeYoung, C.G., Fossella, J.A., Fan, J., Gray, J.R., 2008. Using genetic data in cognitive neuroscience: from growing pains to genuine insights. *Nat. Rev. Neurosci.* 9, 710–720.
- Green, A.E., Kraemer, D.J., DeYoung, C.G., Fossella, J.A., Gray, J.R., 2013. A gene-brain-cognition pathway: prefrontal activity mediates the effect of COMT on cognitive control and IQ. *Cereb. Cortex* 23, 552–559.
- Harmon-Jones, E., Gable, P.A., Peterson, C.K., 2010. The role of asymmetric frontal cortical activity in emotion-related phenomena: a review and update. *Biol. Psychol.* 84, 451–462.
- Hauser, T.U., Iannaccone, R., Walitza, S., Brandeis, D., Brem, S., 2015. Cognitive flexibility in adolescence: neural and behavioral mechanisms of reward prediction error processing in adaptive decision making during development. *Neuroimage* 104, 347–354.
- Hayes, A.F., 2012. PROCESS. A versatile computational tool for observed variable mediation, moderation, and conditional process modeling [White paper]. <http://www.afhayes.com/introduction-to-mediation-moderation-and-conditional-process-analysis.html#process>.
- Hess, C., Reif, A., Strobel, A., Boreatti-Hummer, A., Heine, M., Lesch, K.P., Jacob, C.P., 2009. A functional dopamine-beta-hydroxylase gene promoter polymorphism is associated with impulsive personality styles, but not with affective disorders. *J. Neural Transm.* 116, 121–130.
- Hu, X., Erb, M., Ackermann, H., Martin, J.A., Grodd, W., Reiterer, S.M., 2011. Voxel-based morphometry studies of personality: issue of statistical model specification—effect of nuisance covariates. *Neuroimage* 54, 1994–2005.
- Hurd, Y.L., Suzuki, M., Sedvall, G.C., 2001. D1 and D2 dopamine receptor mRNA expression in the whole hemisphere sections of the human brain. *J. Chem. Neuroanat.* 22, 127–137.
- Hyde, K.L., Lerch, J., Norton, A., Forgeard, M., Winner, E., Evans, A.C., Schlaug, G., 2009. Musical training shapes structural brain development. *J. Neurosci.* 29, 3019–3025.
- Iidaka, T., Matsumoto, A., Ozaki, N., Suzuki, T., Iwata, N., Yamamoto, Y., Okada, T., Sadato, N., 2006. Volume of left amygdala subregion predicted temperamental trait of harm avoidance in female young subjects. A voxel-based morphometry study. *Brain Res.* 1125, 85–93.
- Instanes, J.T., Haavik, J., Halmoy, A., 2013. Personality traits and comorbidity in adults with ADHD. *J. Atten. Disord.* <http://dx.doi.org/10.1177/1087054713511986> [Epub ahead of print].
- Jacob, C., Gross-Lesch, S., Jans, T., Geissler, J., Reif, A., Dempfle, A., Lesch, K.P., 2014. Internalizing and externalizing behavior in adult ADHD. *Atten. Defic. Hyperact. Disord.* 6, 101–110.
- Johnson, A.D., Handsaker, R.E., Pulit, S.L., Nizzari, M.M., O'Donnell, C.J., de Bakker, P.I., 2008. SNP: a web-based tool for identification and annotation of proxy SNPs using HapMap. *Bioinformatics* 24, 2938–2939.
- Kaag, A.M., Crunelle, C.L., van Wingen, G., Homberg, J., van den Brink, W., Reneman, L., 2014. Relationship between trait impulsivity and cortical volume, thickness and surface area in male cocaine users and non-drug using controls. *Drug Alcohol Depend.* 144, 210–217.
- Kaasinen, V., Aalto, S., Nagren, K., Rinne, J.O., 2004. Insular dopamine D2 receptors and novelty seeking personality in Parkinson's disease. *Mov. Disord.* 19, 1348–1351.
- Kaufman, S., Friedman, S., 1965. Dopamine-beta-hydroxylase. *Pharmacol. Rev.* 17, 71–100.
- Kazantseva, A., Gaysina, D., Kutlumbetova, Y., Kanzararova, R., Malykh, S., Lobaskova, M., Khusnutdinova, E., 2014. Brain derived neurotrophic factor gene (BDNF) and personality traits: the modifying effect of season of birth and sex. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 56C, 58–65.
- Kim, S.W., Grant, J.E., 2001. Personality dimensions in pathological gambling disorder and obsessive-compulsive disorder. *Psychiatry Res.* 104, 205–212.
- Kristensen, A.S., Mortensen, E.L., Mors, O., 2009. The association between bodily anxiety symptom dimensions and the scales of the Revised NEO Personality Inventory and the Temperament and Character Inventory. *Compr. Psychiatry* 50, 38–47.
- Krmpotich, T.D., Tregellas, J.R., Thompson, L.L., Banich, M.T., Klenk, A.M., Tanabe, J.L., 2013. Resting-state activity in the left executive control network is associated with behavioral approach and is increased in substance dependence. *Drug Alcohol Depend.* 129, 1–7.
- Lee, T.M., Chan, C.C., Han, S.H., Leung, A.W., Fox, P.T., Gao, J.H., 2008. An event-related fMRI study on risk taking by healthy individuals of high or low impulsiveness. *Neurosci. Lett.* 438, 138–141.
- Lei, X., Chen, C., Xue, F., He, Q., Liu, Q., Moyzis, R.K., Xue, G., Cao, Z., Li, J., Li, H., Zhu, B., Liu, Y., Hsu, A.S., Dong, Q., 2014. Fiber connectivity between the striatum and cortical and subcortical regions is associated with temperaments in Chinese males. *Neuroimage* 89, 226–234.
- Levin, E.Y., Levenberg, B., Kaufman, S., 1960. The enzymatic conversion of 3,4-dihydroxyphenylethylamine to norepinephrine. *J. Biol. Chem.* 235, 2080–2086.
- Long, H., Liu, B., Hou, B., Wang, C., Li, J., Qin, W., Wang, D., Zhou, Y., Kendrick, K.M., Yu, C., Jiang, T., 2013. The long rather than the short allele of 5-HTTLPR predisposes Han Chinese to anxiety and reduced connectivity between prefrontal cortex and amygdala. *Neurosci. Bull.* 29, 4–15.
- Lyall, A.E., Shi, F., Geng, X., Woolson, S., Li, G., Wang, L., Hamer, R.M., Shen, D., Gilmore, J.H., 2014. Dynamic development of regional cortical thickness and surface area in early childhood. *Cereb. Cortex.* <http://dx.doi.org/10.1093/cercor/bhu027> [Epub ahead of print].
- Lyoo, I.K., Lee, D.W., Kim, Y.S., Kong, S.W., Kwon, J.S., 2001. Patterns of temperament and character in subjects with obsessive-compulsive disorder. *J. Clin. Psychiatry* 62, 637–641.
- Meyer-Lindenberg, A., Weinberger, D.R., 2006. Intermediate phenotypes and genetic mechanisms of psychiatric disorders. *Nat. Rev. Neurosci.* 7, 818–827.
- Milivojevic, D., Milovanovic, S.D., Jovanovic, M., Svrakic, D.M., Svrakic, N.M., Svrakic, S.M., Cloninger, C.R., 2012. Temperament and character modify risk of drug addiction and influence choice of drugs. *Am. J. Addict.* 21, 462–467.
- Mohr, P.N., Biele, G., Heekeren, H.R., 2010. Neural processing of risk. *J. Neurosci.* 30, 6613–6619.
- Montag, C., Markett, S., Basten, U., Stelzel, C., Fiebach, C., Canli, T., Reuter, M., 2010. Epistasis of the DRD2/ANKK1 Taq Ia and the BDNF Val66Met polymorphism impacts novelty seeking and harm avoidance. *Neuropsychopharmacology* 35, 1860–1867.
- Munafo, M.R., Yalcin, B., Willis-Owen, S.A., Flint, J., 2008. Association of the dopamine D4 receptor (DRD4) gene and approach-related personality traits: meta-analysis and new data. *Biol. Psychiatry* 63, 197–206.
- Mustapic, M., Maihofer, A.X., Mahata, M., Chen, Y., Baker, D.G., O'Connor, D.T., Nievergelt, C.M., 2014. The catecholamine biosynthetic enzyme dopamine beta-hydroxylase (DBH): first genome-wide search positions trait-determining variants acting additively in the proximal promoter. *Hum. Mol. Genet.* <http://dx.doi.org/10.1093/hmg/ddu332> [Epub ahead of print].
- Naqvi, N.H., Bechara, A., 2009. The hidden island of addiction: the insula. *Trends Neurosci.* 32, 56–67.
- Naqvi, N.H., Rudrauf, D., Damasio, H., Bechara, A., 2007. Damage to the insula disrupts addiction to cigarette smoking. *Science* 315, 531–534.
- O'Connor, D.T., Cervenka, J.H., Stone, R.A., Levine, G.L., Parmer, R.J., Franco-Bourland, R.E., Madrazo, I., Langlais, P.J., Robertson, D., Biaggioni, I., 1994. Dopamine beta-hydroxylase immunoreactivity in human cerebrospinal fluid: properties, relationship to central noradrenergic neuronal activity and variation in Parkinson's disease and congenital dopamine beta-hydroxylase deficiency. *Clin. Sci. (Lond.)* 86, 149–158.
- Panizzon, M.S., Fennema-Notestine, C., Eyer, L.T., Jernigan, T.L., Prom-Wormley, E., Neale, M., Jacobson, K., Lyons, M.J., Grant, M.D., Franz, C.E., Xian, H., Tsuang, M., Fischl, B., Seidman, L., Dale, A., Kremen, W.S., 2009. Distinct genetic influences on cortical surface area and cortical thickness. *Cereb. Cortex* 19, 2728–2735.
- Preuss, U.W., Wurst, F.M., Ridinger, M., Rujescu, D., Fehr, C., Koller, G., Bondy, B., Wodarz, N., Soyka, M., Zill, P., 2013. Association of functional DBH genetic variants with alcohol dependence risk and related depression and suicide attempt phenotypes: results from a large multicenter association study. *Drug Alcohol Depend.* 133, 459–467.
- Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., de Bakker, P.I., Daly, M.J., Sham, P.C., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* 81, 559–575.
- Rakic, P., 1988. Specification of cerebral cortical areas. *Science* 241, 170–176.
- Rakic, P., 2009. Evolution of the neocortex: a perspective from developmental biology. *Nat. Rev. Neurosci.* 10, 724–735.
- Richter, J., Brandstrom, S., 2009. Personality disorder diagnosis by means of the Temperament and Character Inventory. *Compr. Psychiatry* 50, 347–352.
- Richter-Schmidinger, T., Alexopoulos, P., Horn, M., Maus, S., Reichel, M., Rhein, C., Lewczuk, P., Sidiropoulos, C., Kneib, T., Pernecky, R., Doerfler, A., Kornhuber, J., 2011. Influence of brain-derived neurotrophic-factor and apolipoprotein E genetic variants on hippocampal volume and memory performance in healthy young adults. *J. Neural Transm.* 118, 249–257.
- Rimol, L.M., Nesvag, R., Hagler Jr., D.J., Bergmann, O., Fennema-Notestine, C., Hartberg, C.B., Haukvik, U.K., Lange, E., Pung, C.J., Server, A., Melle, I., Andreassen, O.A., Agartz, I., Dale, A.M., 2012. Cortical volume, surface area, and thickness in schizophrenia and bipolar disorder. *Biol. Psychiatry* 71, 552–560.
- Roy, A., Brockington, K., 1987. Plasma dopamine-beta-hydroxylase in depressed patients and controls. *Neuropsychobiology* 18, 57–59.
- Schilling, C., Kuhn, S., Paus, T., Romanowski, A., Banaschewski, T., Barbot, A., Barker, G.J., Bruhl, R., Buchel, C., Conrod, P.J., Dalley, J.W., Flor, H., Ittermann, B., Ivanov, N., Mann, K., Martinot, J.L., Nees, F., Rietschel, M., Robbins, T.W., Smolka, M.N., Strohle, A., Kathmann, N., Garavan, H., Heinz, A., Schumann, G., Gallinat, J., 2013a. Cortical thickness of superior frontal cortex predicts impulsiveness and perceptual reasoning in adolescence. *Mol. Psychiatry* 18, 624–630.

- Schilling, C., Kuhn, S., Romanowski, A., Banaschewski, T., Barbot, A., Barker, G.J., Bruhl, R., Buchel, C., Charlet, K., Conrod, P.J., Czech, K., Dalley, J.W., Flor, H., Hake, I., Ittermann, B., Ivanov, N., Mann, K., Ludemann, K., Martinot, J.L., Palafox, C., Paus, T., Poline, J.B., Reuter, J., Rietschel, M., Robbins, T.W., Smolka, M.N., Strohle, A., Walaszek, B., Kathmann, N., Schumann, G., Heinz, A., Garavan, H., Gallinat, J., 2013b. Common structural correlates of trait impulsiveness and perceptual reasoning in adolescence. *Hum. Brain Mapp.* 34, 374–383.
- Schroeder, J.P., Cooper, D.A., Schank, J.R., Lyle, M.A., Gaval-Cruz, M., Ogbonmwan, Y.E., Pozdeyev, N., Freeman, K.G., Iuvone, P.M., Edwards, G.L., Holmes, P.V., Weinschenker, D., 2010. Disulfiram attenuates drug-primed reinstatement of cocaine seeking via inhibition of dopamine beta-hydroxylase. *Neuropsychopharmacology* 35, 2440–2449.
- Smith, C.D., Chebrou, H., Wekstein, D.R., Schmitt, F.A., Markesbery, W.R., 2007. Age and gender effects on human brain anatomy: a voxel-based morphometric study in healthy elderly. *Neurobiol. Aging* 28, 1075–1087.
- Sugiura, M., Kawashima, R., Nakagawa, M., Okada, K., Sato, T., Goto, R., Sato, K., Ono, S., Schormann, T., Zilles, K., Fukuda, H., 2000. Correlation between human personality and neural activity in cerebral cortex. *Neuroimage* 11, 541–546.
- Suhara, T., Yasuno, F., Sudo, Y., Yamamoto, M., Inoue, M., Okubo, Y., Suzuki, K., 2001. Dopamine D2 receptors in the insular cortex and the personality trait of novelty seeking. *Neuroimage* 13, 891–895.
- Sutton, S.K., Davidson, R.J., 1997. Prefrontal brain asymmetry: a biological substrate of the behavioral approach and inhibition systems. *Psychol. Sci.* 8.
- Tiu, S.C., Yew, D.T., Chan, W.Y., 2003. Development of the human cerebral cortex: a histochemical study. *Prog. Histochem. Cytochem.* 38, 3–149.
- van Honk, J., Schutter, D.J., 2006. From affective valence to motivational direction: the frontal asymmetry of emotion revised. *Psychol. Sci.* 17, 963–965.
- Van Schuerbeek, P., Baeken, C., De Raedt, R., De Mey, J., Luypaert, R., 2011. Individual differences in local gray and white matter volumes reflect differences in temperament and character: a voxel-based morphometry study in healthy young females. *Brain Res.* 1371, 32–42.
- Vuoksimaa, E., Panizzon, M.S., Chen, C.H., Fiecas, M., Eyer, L.T., Fennema-Notestine, C., Hagler, D.J., Fischl, B., Franz, C.E., Jak, A., Lyons, M.J., Neale, M.C., Rinker, D.A., Thompson, W.K., Tsuang, M.T., Dale, A.M., Kremen, W.S., 2014. The genetic association between neocortical volume and general cognitive ability is driven by global surface area rather than thickness. *Cereb. Cortex.* <http://dx.doi.org/10.1093/cercor/bhu018> [Epub ahead of print].
- Wing, V.C., Payer, D.E., Houle, S., George, T.P., Boileau, I., 2015. Measuring cigarette smoking-induced cortical dopamine release: a [(11)C]FLB-457 PET study. *Neuropsychopharmacology* 40, 1417–1427.
- Winkler, A.M., Kochunov, P., Blangero, J., Almasy, L., Zilles, K., Fox, P.T., Duggirala, R., Glahn, D.C., 2010. Cortical thickness or grey matter volume? The importance of selecting the phenotype for imaging genetics studies. *Neuroimage* 53, 1135–1146.
- Yew, D.T., Luo, C.B., Shen, W.Z., Chow, P.H., Zheng, D.R., Yu, M.C., 1995. Tyrosine hydroxylase- and dopamine-beta-hydroxylase-positive neurons and fibres in the developing human cerebellum—an immunohistochemical study. *Neuroscience* 65, 453–461.
- Zabetian, C.P., Anderson, G.M., Buxbaum, S.G., Elston, R.C., Ichinose, H., Nagatsu, T., Kim, K.S., Kim, C.H., Malison, R.T., Geleter, J., Cubells, J.F., 2001. A quantitative-trait analysis of human plasma-dopamine beta-hydroxylase activity: evidence for a major functional polymorphism at the DBH locus. *Am. J. Hum. Genet.* 68, 515–522.