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COMT Val^{158}*Met* × *SLC6A4* 5-HTTLPR interaction impacts on gray matter volume of regions supporting emotion processing

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There have been several reports on the association between the *Val*^{158}*Met* genetic polymorphism of the catechol-*O*-methyltransferase (*COMT*) gene, as well as the serotonin transporter-linked polymorphic region (*5-HTTLPR*) of the serotonin transporter gene (*SLC6A4*), and frontolimbic region volumes, which have been suggested to underlie individual differences in emotion processing or susceptibility to emotional disorders. However, findings have been somewhat inconsistent. This study used diffeomorphic anatomic registration through exponentiated Lie algebra (DARTEL) whole-brain voxel-based morphometry to study the genetic effects of *COMT Val*^{158}*Met* and *SLC6A4* 5-HTTLPR, as well as their interaction, on the regional gray matter volumes of a sample of 91 healthy volunteers. An interaction of *COMT Val*^{158}*Met* × *SLC6A4* 5-HTTLPR genotypes with gray matter volume was found in bilateral parahippocampal gyrus, amygdala, hippocampus, vermis of cerebellum and right putamen/insula. In particular, the gray matter volume in these regions was smaller in individuals who were both *COMT-Met* and 5-HTTLPR-*S* carriers, or both *COMT-Val* and 5-HTTLPR-*L* homozygotes, as compared with individuals with intermediate combinations of alleles. The interaction of *COMT Val*^{158}*Met* and *SLC6A4* 5-HTTLPR adds to the understanding of individual differences in emotion processing.

Keywords: catechol-*O*-methyltransferase; genetic interaction; gray matter; serotonin transporter gene; serotonin transporter-linked polymorphic region; voxel-based morphometry

INTRODUCTION

A number of studies in the last 10 years have associated some genetic polymorphisms with increased vulnerability to depressive disorders. In particular, the short allele (*S*) of the serotonin transporter-linked polymorphic region (5-HTTLPR) of the serotonin transporter gene (*SLC6A4*), as well as the *Val* allele of the *Met*^{158}*Val* polymorphism of the catechol-*O*-methyltransferase (*COMT*) gene, have been strongly associated with a vulnerability to major depressive disorder (MDD) (Caspi *et al.*, 2003; Kendler *et al.*, 2005; Mandelli *et al.*, 2007; Conway *et al.*, 2010; Aberg *et al.*, 2011; Karg *et al.*, 2011).

Structural neuroimaging genetic studies looking for intermediate phenotypes of depression have also demonstrated brain volume differences associated with the above genes. For example, several groups (Canli *et al.*, 2005; Pezawas *et al.*, 2005; Frodl *et al.*, 2008; Selvaraj *et al.*, 2010) reported that carriers of 5-HTTLPR-*S* compared with long allele (*L*) had smaller gray matter volumes in the amygdala, hippocampus, anterior cingulate cortex, superior, middle and inferior frontal gyri, dorsolateral prefrontal cortex and superior temporal gyrus. This evidence is in agreement with the findings of smaller volumes of hippocampal/temporolimbic structures in people predisposed to MDD, e.g. the offspring of depressed patients (Chen *et al.*, 2010) or the monozygotic twins of patients (Baare *et al.*, 2010). In contrast, studies of *COMT Val*^{158}*Met* effect on brain structure found increased volume of hippocampus in *COMT-Met* compared with *Val* allele carriers (Taylor *et al.*, 2007; Cerasa *et al.*, 2008). However, it must be highlighted that other important studies have failed to detect any genetic effects of *SLC6A4* 5-HTTLPR or *COMT Val*^{158}*Met* on gray matter volumes (Zinkstok *et al.*, 2006; Dutt *et al.*, 2009).

It is known that genetic factors predisposing toward complex syndromes such as MDD often demonstrate an interaction, even in the absence of any main effects (Grigorenko *et al.*, 2003). We have recently reported an interaction of *COMT Val*^{158}*Met* and *SLC6A4* 5-HTTLPR with the Granger effective connectivity within the emotion processing circuit in healthy individuals (Surguladze *et al.*, 2012). This study tested the hypothesis that an interaction between *COMT Val*^{158}*Met* and *SLC6A4* 5-HTTLPR genotypes would be associated with the size of gray matter in brain regions involved in emotion processing. To this end, the effects of *COMT Val*^{158}*Met*, *SLC6A4* 5-HTTLPR and their interaction on brain gray matter volume were assessed using structural MRI data acquired on a large sample of healthy volunteers and applying advanced voxel-based morphometry algorithms, namely: the 'new segmentation' and 'diffeomorphic anatomic registration through exponentiated Lie algebra' (DARTEL) method, which provides enhanced accuracy (Ashburner, 2007).

METHODS

Participants

The participants were 91 right-handed healthy Caucasian individuals (Table 1) with no family history of psychiatric disorder. Exclusion

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The last two authors contributed equally to this study.

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Table 1 Characteristics of the healthy individuals participating in this study.

	All participants	*Val* and *L′* homozygotes	*Val* homozygotes but *S′* carriers	*Met* carriers but *L′* homozygotes	Both *Met* and *S′* carriers
Sample size	91	7	13	19	52
Sex (% males)	51	71	62	32	52
Age (years)[a]	33 ± 9	36 ± 13	34 ± 8	33 ± 9	31 ± 9
BDI[a]	4.4 ± 5	5.8 ± 4	3.5 ± 4	5.6 ± 8	3.9 ± 4
Harm avoidance[a]	90 ± 19	92 ± 28	88 ± 19	85 ± 18	91 ± 18
Novelty seeking[a]	106 ± 13	104 ± 12	108 ± 14	106 ± 13	106 ± 13
Persistence[a]	123 ± 18	115 ± 18	120 ± 22	128 ± 17	123 ± 18
Reward dependence[a]	104 ± 16	92 ± 21	100 ± 13	107 ± 15	106 ± 16

[a]Values are expressed as mean ± SD.

criteria were current or past psychiatric diagnosis as established by the Structured Clinical Interview for DSM-IV (SCID I) (APA, 1994). The study was approved by the Ethics Committee of the Institute of Psychiatry, King's College London. After a complete description of the study to the subjects, written informed consent was obtained before the experiments began.

Participants underwent an evaluation of depressive symptoms with the Beck Depression Inventory (BDI) II (Beck *et al.*, 1996). The mean total BDI on the day of scanning was 3.1 ± 3.7 (below the cut-off level of 'minimal depression'), though one participant scored 18 (i.e. within the range of 'mild depression'). Participants also completed the 'harm avoidance', 'novelty seeking', 'reward dependence' and 'persistence' scales of Cloninger's Temperament and Character Inventory (TCI; Cloninger *et al.*, 1993), whose dimensions have been shown to be genetically modulated (Heath *et al.*, 1994).

Genotyping

DNA was extracted from cheek swabs using standard procedures. The genotype of the *COMT Val*^{158}*Met* (rs4680) single nucleotide polymorphism (SNP) was determined by allelic discrimination assay (C_25746809_50) based on fluorogenic 5′ nuclease activity: a TaqMan SNP genotyping assay was performed the ABI Prism 7900HT and analyzed with Sequence Detection System software according to the manufacturer's instructions (Applied Biosystems, Warrington, UK). Twenty participants were found to be *Val* homozygotes and the remaining 71 found to be *Met* carriers.

To determine the genotypes of the *SLC6A4* 5-HTTLPR insertion/deletion and of the rs25531 G/A SNP, a modified version of the protocol described by Wendland *et al.* (2006) was used. The 5′-TCCTCCGCTTTGGCGCCTCTTCC-3′ forward and 5′-TGGGGG TTGCAGGGGAGATCCTG-3′ reverse primers were used (Operon, Huntsville, AT) that amplify a 469 (short or S allele) and 512 (long or L allele) product. In a total volume of 20 μl, 25 ng of genomic DNA were amplified in the presence of 1× polymerase chain reaction (PCR) Buffer (Qiagen) and oligonucleotide primers. A combination of Q solution (Qiagen), c7–dGTP Q (ROCHE) and AmpliTaq Gold (ABI) were used to facilitate successful genotyping. Q (5×) solution improves suboptimal PCR systems caused by templates that have a high degree of secondary structure or that GC rich. The incorporation of c^7–dGTP in the dNTP mix (the concentration mix contained 0.2 mM of A, C, T and 0.1 mM of G and c^7) can eliminate spurious GC–hydrogen bonding and relax secondary structures and AmpliTaq Gold (1.25 U), provides increased sensitivity, specificity and yield over conventional PCR techniques. $MgCl_2$ (ABI) of 1.8 mM was added to the final mix. Thermal cycling consisted of 15 min of initial denaturation at 95°C followed by 42 cycles of 94°C (30 s) 68°C (90 s) and 72°C (60 s) each with a final extension step of 10 min at 72°C. PCR product were loaded onto 3% agarose gel run for 1 hour at 120 V in 1× TBE and visualized by ethidium bromide (Sigma-Aldrich, St Louis, MO). Ten mocroliters of the remaining PCR product was digested for 12 h at 37°C with MSP1 (5 U/μl) (New England Biolabs, Ipswich, MA) which cuts the 5′-C/CGG-3′ sequence. Digestion with MSP1 resulted in the following fragments: a 469 bp product (short uncut) representing the SA allele; a 402 bp + 67 bp product (short cut) representing the SG allele; a 512 bp product (long uncut) representing the LA allele and a 402 bp + 110 bp product (long cut) representing the LG allele. This is an improved protocol for the amplification of 5-HTTLPR which resulted in clear and easily distinguishable bands. As the *Long* allele behaves like the *Short* allele in the presence of the rs25531 *A*/*G* substitution, therefore *SLC6A4* 5-HTTLPR polymorphisms were recoded as *L′* (*Long A*) and *S′* (*Short A*, *Short G* or *Long G*) (Parsey *et al.*, 2006). Twenty-six participants were found to be *L′* homozygotes, and the remaining 65 were found to be *S′* carriers.

Conditional frequencies of *COMT-Met* carriers and 5-HTTLPR-*S′* carriers were found to be highly balanced in the sample ($\chi^2 = 0.1939$, $df = 1$, $P = 0.660$). The frequency of *COMT-Met* carriers in the subsample of 5-HTTLPR-*L′* homozygotes (73%, 19 out of 26 individuals) was very similar to the frequency of *COMT-Met* carriers in the subsample of 5-HTTLPR-*S′* carriers (80%, 52 out of 65 individuals). Similarly, the frequency of 5-HTTLPR-*S′* carriers in the subsample of *COMT-Val* homozygotes (65%, 13 out of 20 individuals) was very similar to the frequency of 5-HTTLPR-*S′* carriers in the subsample of *COMT-Met* carriers (73%, 52 out of 71 individuals).

Finally, cross-tabulation of the *COMT Val*^{158}*Met* and *SLC6A4* 5-HTTLPR alleles yielded four genetic groups with similar age and sex distribution: (i) individuals who were both *COMT-Val* and 5-HTTLPR-*L′* homozygotes; (ii) individuals who were *COMT-Val* homozygotes but 5-HTTLPR-*S′* carriers; (iii) individuals who were *COMT-Met* carriers but 5-HTTLPR-*L′* homozygotes; and (iv) individuals who were both *COMT-Met* and 5-HTTLPR-*S′* carriers. As shown in Table 1, the sample size of some of the genetic groups was small, a condition which does not increase the likelihood of false positive findings (Friston, 2012) but may decrease the power to detect small differences between groups. However, it must be noted that the power of statistical comparisons depends on the combined sample size of the different groups, and a post hoc power analysis revealed that the power to detect interaction effects in this study was equivalent to the power of a standard *t* test with 28 individuals per group.

There were no significant differences of age or female/male distribution related to the genetic groups. Age: *COMT Val*^{158}*Met* × *SLC6A4* 5-HTTLPR two-factorial analysis of variance (ANOVA) *P* values > 0.05; female/male distribution: *COMT Val*^{158}*Met* × *SLC6A4* 5-HTTLPR interaction log-linear model *P* values > 0.05. As detailed later, the age and sex variables will be included as covariates in the statistical analysis to decrease the residual variance and thus increase the power to detect differences between groups. Similarly, there were no significant differences in the BDI level and the four TCI dimension scores between the genetic groups (*COMT Val*^{158}*Met* × *SLC6A4* 5-HTTLPR two-factorial ANOVA *P* values > 0.05), with the exception of a trend toward higher 'reward dependence' TCI score in *COMT-Met* carriers (106 ± 16 vs. 97 ± 16 in *Val* homozygotes; *t* test uncorrected *P* value = 0.04, corrected *P* value > 0.05).

Magnetic resonance imaging

Scanning was performed on a General Electric Signa 3T scanner at the Institute of Psychiatry, acquiring 196 T1-weigthed fast spoiled gradient echo coronal slices (TR/TE/TI 7.1/2.8/450 ms, flip angle 20°, FOV 280 mm, 256 × 256 matrix, voxel size 1.1 × 1.1 × 1.1 mm^3).

Preprocessing steps with SPM8 (Welcome Trust Centre for Neuroimaging, London) were as follows: (i) manual realignment and centering at the anterior commissure; (ii) segmentation into gray matter, white matter, cerebrospinal fluid and other tissues; (iii) iterative creation of a study-specific template; (iv) normalization of gray matter images to MNI space through the final template of the series and the flow fields; and (v) smoothing by an isotropic Gaussian kernel. Following DARTEL defaults, we conducted the latter step with an 8 mm (rather than 12 mm) FWHM kernel. This narrow kernel is probably recommended because high-resolution normalization algorithms such as DARTEL are thought to require a lower degree of smoothing, and narrow kernels have been found to be more sensitive to detect abnormalities in small brain structures such as the amygdala (Uchida *et al.*, 2008). Finally, individual volumes were voxel-wise scaled by the Jacobian determinant of their transformation to prevent volume differences due to spatial deformations.

Analysis

Effects of *COMT Val^{158}Met* and *SLC6A4* 5-HTTLPR were assessed by *t* tests comparing whole brain regional gray matter volume, e.g. between *COMT-Met* carriers and *COMT-Val* homozygotes, with age and sex as covariates. The interaction between *COMT Val^{158}Met* and *SLC6A4* 5-HTTLPR was assessed with a *COMT Val^{158}Met* × *SLC6A4* 5-HTTLPR two-factorial analysis of covariance (ANCOVA), with age and sex as covariates. All analyses were proportionally scaled by global gray matter. Statistical significance was assessed at the cluster level and based on the Gaussian random fields' theory (Worsley and Friston, 1995). Specifically, clusters of gray matter volume difference were first defined using a voxel threshold of $P < 0.001$ and a size threshold of >100 voxels, but only those clusters with a false discovery rate (FDR) < 0.05 were considered as statistically significant to correct for multiple comparisons. Peak coordinates were Lancaster-transformed to Talairach space with the SDM online utilities (www.sdmproject.com/utilities) (Lancaster *et al.*, 2007; Radua and Mataix-Cols, 2009). The gray matter volume of each cluster in each individual native space was then estimated by summing the values of its voxels and scaled by global gray matter—note that the value of a voxel in a modulated image is informative of its volume in native space. Potential associations between these volumes and mood and personality characteristics (BDI and TCI) were assessed with Pearson correlations.

A region of interest (ROI) analysis was also conducted of the amygdala and hippocampus, based on consistent findings of volume reductions in these regions associated with MDD (see meta-analyses; Videbech and Ravnkilde, 2004; Cole *et al.*, 2011; Kempton *et al.*, 2011; Arnone *et al.*, 2012; Bora *et al.*, 2012) or with risk of developing MDD (Chen *et al.*, 2010). ROIs were defined according to the Harvard–Oxford atlas (www.fmrib.ox.ac.uk/fsl/data/atlas-descriptions.html#ho), which showed a good overlap with the study-specific template. The gray matter volume of each ROI was estimated in each individual as the sum of the values of its voxels and scaled by global gray matter. ROI volumes were then compared across genetic groups using the *t* tests and ANCOVA described earlier, with Bonferroni–Holm correction for multiple comparisons. Holm's modification of the Bonferroni method gives strong control of the family-wise error rate, whilst it has increased power and is valid under arbitrary assumptions (Holm, 1979).

RESULTS

Voxel-based morphometry

There was a significant interaction of *COMT Val^{158}Met* × *SLC6A4* 5-HTTLPR that had effects on the gray matter volume of bilateral parahippocampal gyrus, cerebellar vermis and right putamen/insula (all corrected $P \leq 0.030$). This interaction was accounted for by 8.33% smaller gray matter volume in the clusters of significant interaction in individuals who were both *COMT-Met* and 5-HTTLPR-*S′* carriers, or both *COMT-Val* and 5-HTTLPR-*L′* homozygotes, as compared with individuals with any other combinations of alleles (Table 2 and Figure 1).

As regards to the main effects, *COMT Val^{158}Met* voxel-based ANCOVA demonstrated increased gray matter volume in a small part of right angular gyrus in *COMT-Met* carriers (peak Talairach coordinate: 52, −62, 34; cluster *P* value = 0.025), though this finding was not observed in the voxel-based *COMT Val^{158}Met* × *SLC6A4* 5-HTTLPR two-factorial voxel-based ANCOVA and thus was not further considered. No statistically significant main effects of *SLC6A4* 5-HTTLPR were detected in the *SLC6A4* 5-HTTLPR voxel-based ANCOVA.

The *COMT Val^{158}Met* × *SLC6A4* 5-HTTLPR interactions described earlier could also be observed when the volume of the clusters found in the earlier mentioned interaction analysis was separately analyzed for male and female individuals, and for younger and older individuals according to median age (<30 years vs. ≥30 years) with *COMT Val^{158}Met* × *SLC6A4* 5-HTTLPR two-factorial ANOVAs. Specifically, the pattern of smaller gray matter volume in individuals who were both *COMT-Met* and 5-HTTLPR-*S′* carriers, or both *COMT-Val* and 5-HTTLPR-*L′* homozygotes, was observed in each of the brain regions in each of the four demographic groups, with the ANOVA interaction term achieving statistical significance in all cases—with the exception of right parahippocampal cluster in younger participants and left parahippocampal cluster in female participants, where only non-significant trends were detected ($P = 0.080$ and 0.103, respectively). There was no significant correlation between the cluster volumes detected in the interaction analysis and the BDI or TCI measures.

ROI analysis

The interaction effect of *COMT Val^{158}Met* and *SLC6A4* 5-HTTLPR on hippocampus and amygdala ROIs was of a similar nature to that described earlier (Table 3). In particular, the hippocampal and amygdalar volumes were smaller in the individuals with both *COMT-Met* and 5-HTTLPR-*S′* alleles, or those individuals who were both *COMT-Val* and 5-HTTLPR-*L′* homozygotes, compared with the individuals with any other combinations of alleles.

Finally, we repeated the interaction analyses separately assessing the three genotypes of *COMT Val^{158}Met* and the three genotypes of *SLC6A4* 5-HTTLPR. As shown in Figure 2, amygdalar and hippocampal volumes were found to progressively vary depending on the number of *COMT-Met* alleles (*Val/Val* > *Val/Met* > *Met/Met* in 5-HTTLPR-*S′* carriers; *Met/Met* > *Val/Met* > *Val/Val* in 5-HTTLPR-*L′* homozygotes). Similarly, amygdalar and hippocampal volumes were found to also progressively vary depending on the number of 5-HTTLPR-*S′* alleles (*L′/L′* > *L′/S′* > *S′/S′* in *COMT-Met* carriers; *S′/S′* > *L′/S′* > *L′/L′* in *COMT-Val* homozygotes). However, findings derived from this exploratory, descriptive analysis should be taken with caution given the small sample size of some of the genotype combinations.

DISCUSSION

In this study, a *COMT Val^{158}Met* × *SLC6A4* 5-HTTLPR interaction upon gray matter volume was observed, by which individuals who carried both *COMT-Met* and 5-HTTLPR-*S′* alleles or none, had symmetrical decreases in gray matter volume in bilateral parahippocampal gyrus, cerebellum, right putamen/insula, amygdala and hippocampus,

Table 2 Whole-brain voxel-based analysis of the *COMT Val*^{158}*Met* × *SLC6A4* 5–HTTLPR interaction

	COMT Val^{158}*Met* × *SLC6A4* 5-HTTLPR interaction		Volumes of the clusters[a] (mm^3 in subjects' native space)			
	Talairach coordinate[b]	Cluster *P* value[c]	*Val* and *L′* homozygotes	*Val* homozygotes but *S′* carriers	*Met* carriers but *L′* homozygotes	Both *Met* and *S′* carriers
Left parahippocampal gyrus (743 voxels)[d]	−14, −11, −19	<0.001	1100 ± 109	1212 ± 105	1200 ± 74	1128 ± 77
Right cerebellum (1766 voxels)[d]	11, −43, −8	—[d]	3231 ± 309	3677 ± 305	3635 ± 257	3438 ± 258
Right putamen/insula (1416 voxels)	34, −2, −8	0.003	2404 ± 250	2657 ± 216	2648 ± 196	2470 ± 162
Right parahippocampal gyrus (736 voxels)	13, −11, −17	0.026	1043 ± 76	1138 ± 84	1129 ± 78	1058 ± 63
Vermis cerebelli 6 (1217 voxels)[e]	14, −72, −24	0.030	2438 ± 239	2787 ± 273	2777 ± 213	2646 ± 188

[a]The ANCOVA was conducted using MNI-normalized images and returned a set of clusters. The gray matter volume of each cluster in each individual was then estimated by summing the values of its voxels. Note that the value of a voxel in a modulated image is informative of its volume in native space.
[b]Location of the cluster peak *t*-value of the *COMT Val*^{158}*Met* × *SLC6A4* 5-HTTLPR interaction term of the two factorial analysis of covariance (ANCOVA with age and sex as covariates).
[c]*P* value after FDR-correction for multiple comparisons.
[d]One left parahippocampal/bilateral cerebellar cluster was split for display purposes in this table by slightly increasing the threshold.
[e]Volumes of two contiguous cerebellar clusters were combined in this table.

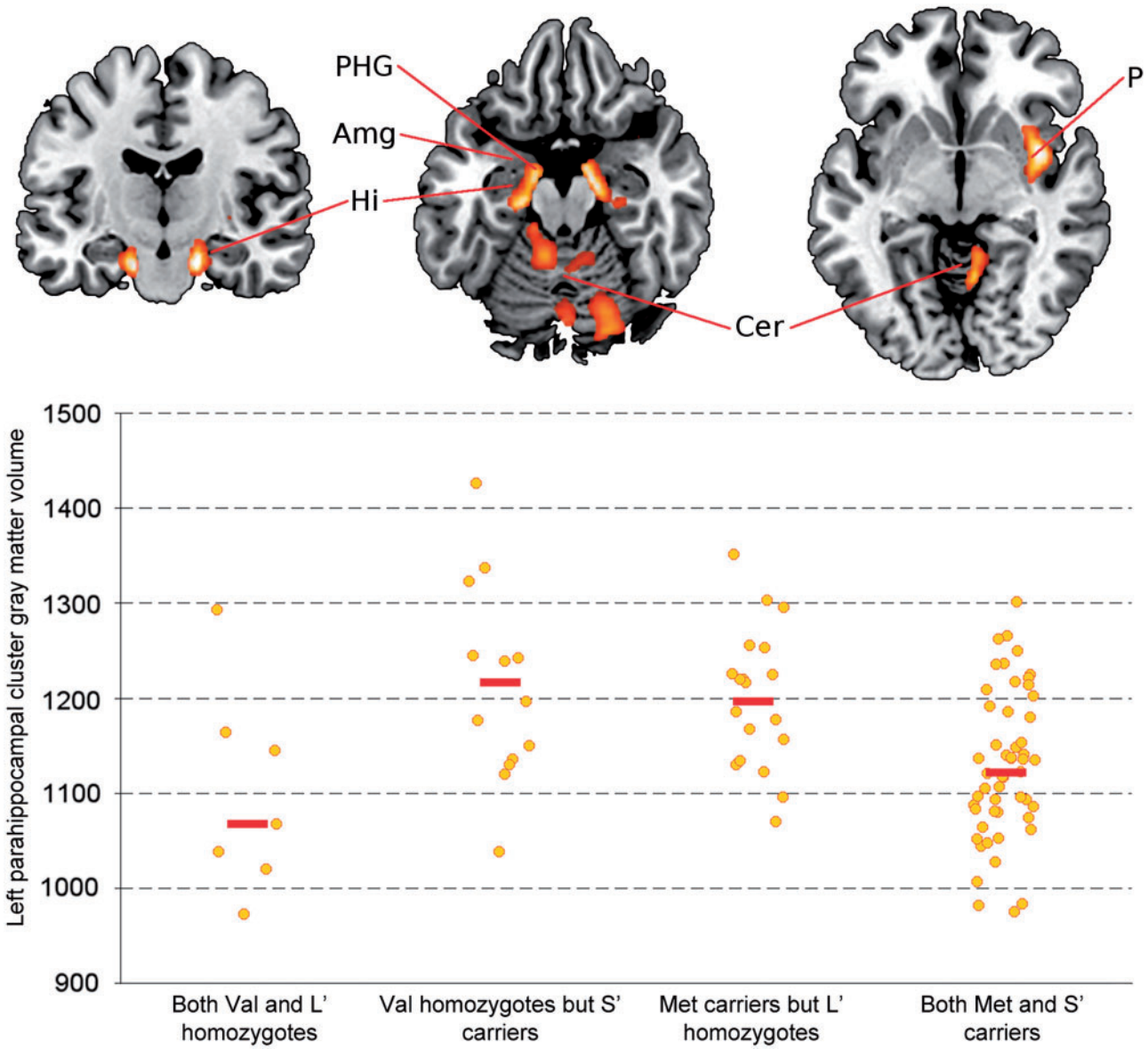

Fig. 1 Interactive effects of *COMT Val*^{158}*Met* and *SLC6A4* 5-HTTLPR on gray matter volume (whole brain analysis). Top: Regions where gray matter volume was smaller in individuals who were both *COMT-Met* and 5-HTTLPR-*S′* carriers, or both *COMT-Val* and 5-HTTLPR-*L′* homozygotes, as compared with those with other combinations of these alleles. Left side of the brain image corresponds to the left side of the brain. Bottom: Individual and genetic group median left parahippocampal cluster gray matter volumes. Amg, amygdala; Cer, cerebellum; Hi, hippocampus; P, putamen; PHG, parahippocampal gyrus. Clusters of gray matter volume difference were defined using a voxel threshold of $P < 0.001$ and a size threshold of >100 voxels, with statistical significance corrected for multiple comparisons (FDR < 0.05). Note that the part inferior of the bar plot has been truncated to 900 for display purposes.

Table 3 ROI analysis of the *COMT Val*^{158}*Met* × *S6C4A5* 5–HTTLPR interaction

	COMT Val^{158}*Met* and *SLC6A4* 5-HTTLPR interaction		Volumes of the regions of interest (mm^3 in subjects' native space)			
	t value[a]	*P* value[b]	*Val* and *L′* homozygotes	*Val* homozygotes but *S′* carriers	*Met* carriers but *L′* homozygotes	Both *Met* and *S′* carriers
Left amygdala	2.31	0.023	2862 ± 350	3055 ± 267	2976 ± 203	2901 ± 214
Right amygdala	3.11	0.006	2745 ± 221	2928 ± 230	2944 ± 212	2813 ± 199
Left hippocampus	2.96	0.008	6173 ± 679	6613 ± 350	6554 ± 360	6336 ± 394
Right hippocampus	3.65	0.002	6045 ± 596	6623 ± 419	6568 ± 394	6324 ± 398

[a]*t* value of the *COMT Val*^{158}*Met* × *SLC6A4* 5-HTTLPR interaction term of the two factorial ANCOVA (with age and sex as covariates).
[b]*P* value after Bonferroni–Holm correction for multiple comparisons. Holm's modification of the Bonferroni method gives strong control of the family-wise error rate whilst it has increased power and is valid under arbitrary assumptions (Holm, 1979).

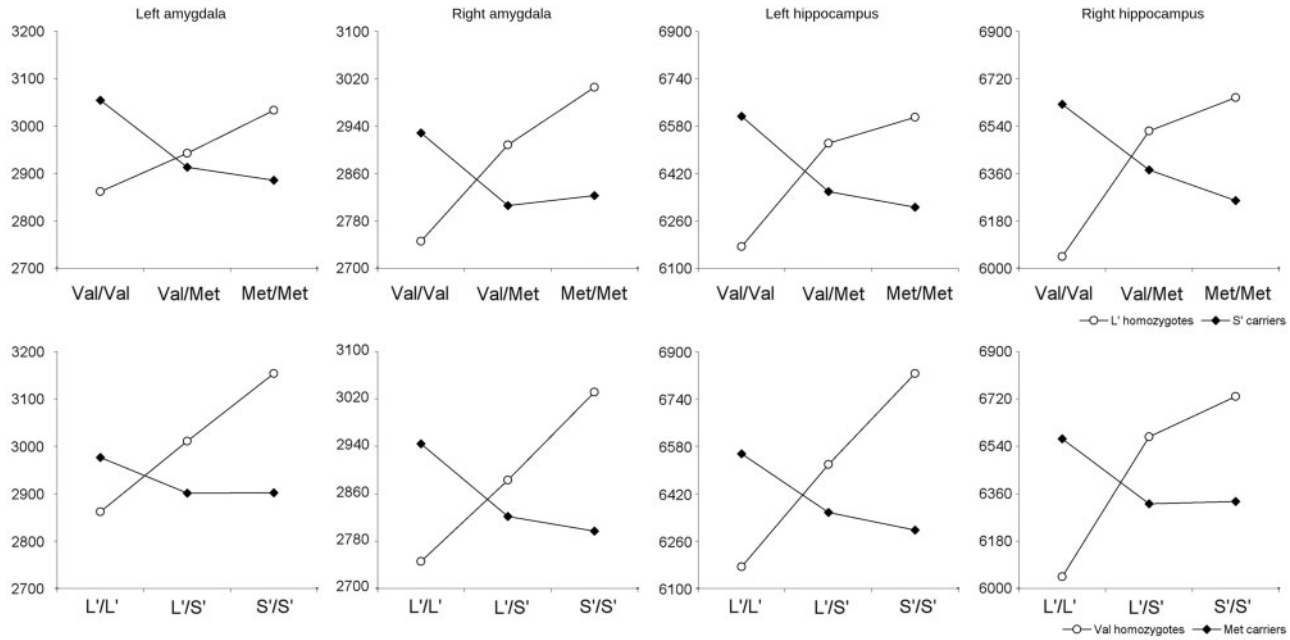

Fig. 2 Interactive effects of *COMT Val*^{158}*Met* and *SLC6A4* 5-HTTLPR on gray matter volume (ROI analysis).

compared with individuals who were homozygous for either (but not simultaneously for both) *Val* or *L′* allele.

These results may help to shed light on the potential reasons behind the diversity of previous findings. For example, in *COMT-Met* carriers, the effects of *SLC6A4* 5-HTTLPR would be such that 5-HTTLPR-*S′* carriers would have smaller gray matter volumes than 5-HTTLPR-*L′* homozygotes, which is in accordance with reports of lower amygdalar volumes in 5-HTTLPR-*S′* carriers (Pezawas *et al.*, 2005). On the other hand, in 5-HTTLPR-*L′* homozygotes, the effects of *COMT Val*^{158}*Met* would be such that *COMT-Met* carriers would have larger gray matter volumes than *COMT-Val* homozygotes. Again, this was the case in previous studies reporting larger hippocampal volumes in *COMT-Met* carriers (Taylor *et al.*, 2007; Cerasa *et al.*, 2008).

However, in samples where the frequencies of *COMT-Met* and 5-HTTLPR-*S′* carriers are balanced, no main effects of one or the other gene should be detected, as it was the case in this study when the effects of *COMT Val*^{158}*Met* and *SLC6A4* 5-HTTLPR were separately assessed. Indeed, studies composed of mainly *COMT-Val* homozygotes or 5-HTTLPR-*S′* carriers could even report results opposite to those published. In the subsample of *COMT-Val* homozygotes, for instance, parahippocampal gray matter volumes were larger (rather than smaller) in 5-HTTLPR-*S′* carriers. Similarly, in the subsample of 5-HTTLPR-*S′* carriers these volumes were smaller (rather than larger) in *COMT-Met* carriers.

The underlying mechanisms of the above interaction need further investigation. The effects of *COMT Val*^{158}*Met* and *SLC6A4* 5-HTTLPR genes on brain structure have been proposed to be related to the role monoamines play in synaptic plasticity during cortical maturation. In particular, dopaminergic innervation has been found to change during the course of cortical development (Rosenberg and Lewis, 1995), an observation which has been proposed to be related to *COMT Val*^{158}*Met*-dependent variation of gray matter volumes (Taylor *et al.*, 2007). There is also evidence that cortical (Gaspar *et al.*, 2003), and hippocampal (Zhang *et al.*, 2006; Choi *et al.*, 2007) development is influenced by serotonin, which could explain the *SLC6A4* 5-HTTLPR-dependent variation of gray matter volumes (Pezawas *et al.*, 2005).

Importantly, the regions identified in this study (hippocampus, amygdala, cerebellum, ventral striatum) have been proposed in a recent study (Glahn *et al.*, 2012) as potential endophenotypes of recurrent MDD. This is in line with previous findings establishing the neuropathology of MDD. For example, meta-analyses (Koolschijn *et al.*, 2009; Kempton *et al.*, 2011; Arnone *et al.*, 2012; Bora *et al.*, 2012) have demonstrated smaller volumes of putamen and hippocampus, and a review (Beyer and Ranga Krishnan, 2002)

has highlighted moderate cerebellar volume reductions in individuals with MDD. The size of the amygdala in MDD has not been found to consistently deviate from normal, however (Kempton *et al.*, 2011).

Findings of this study indicate that both hypo- and hypermetabolism of dopamine and serotonin may be associated with reduced volumes of the brain regions involved in emotion processing. Thus, smaller gray matter volumes were shown in individuals with both *COMT-Met* and 5-HTTLPR-*S′* low activity alleles, and also in individuals homozygous for both *COMT-Val* and 5-HTTLPR-*L′* high activity alleles. It could be thus suggested that both extremes of neurotransmitter metabolism negatively impact on regional brain volume development, resembling the inverted U-shape relationship previously suggested between *COMT-Val* and *COMT-Met* alleles and dopaminergic function (Meyer-Lindenberg *et al.*, 2005). Importantly, the interaction between these alleles on gray matter volumes was very similar to our previous finding regarding effective connectivity measured in the same sample of individuals (Surguladze *et al.*, 2012). Here, effective connectivity in emotion processing neural circuitry was reduced in individuals who carried both *COMT-Met* and 5-HTTLPR-*S′* alleles. Individuals who were both *COMT-Val* and 5-HTTLPR-*L′* homozygotes showed normal connectivity values, though a slight trend could be observed towards reduced connectivity. It is therefore plausible that there may be developmental effects of the interaction of *COMT Val*^{158}*Met* and *SLC6A4* 5-HTTLPR on both gray matter volume and effective connectivity.

It is important to highlight several limitations of this study. First, the sample size of some of the genetic groups was small, a condition which does not increase the likelihood of false positive findings (Friston, 2012) but may decrease the power to detect small differences between groups. However, it must be noted that the power of statistical comparisons depends on the sample size of the different groups and not only on the sample size of the smallest group. Indeed, a simulation work estimated that the power to detect interaction effects with the sample sizes of this study was equivalent to that achieved in a standard *t* test with 28 participants per group. Second, no associations between brain volume and the phenotypes related to emotion processing variance (i.e. TCI and BDI) could be detected. The presence of such an association would have provided external validity to the suggestion of a genetic interaction effect on the regions involved in emotional processes. Thus far, this proposal is supported by the existing literature indicating the role of amygdala, hippocampus, cerebellum and putamen in the earlier mentioned processes (Koolschijn *et al.*, 2009; Kempton *et al.*, 2011; Arnone *et al.*, 2012; Bora *et al.*, 2012). Further studies involving larger sample sizes are warranted to explore possible associations between individual measures of emotion processing, genetic interaction effects and regional brain volumes.

To sum up, the results of this study showed interaction effects of *COMT Val*^{158}*Met* and *SLC6A4* 5-HTTLPR polymorphisms on the gray matter volumes of structures involved in emotion processing in healthy individuals. These effects may underlie the individual differences in emotion processing, and potentially inform about the mechanisms of susceptibility to MDD. These findings also help to disentangle some of the inconsistencies in the findings of previous studies that separately assessed the effects of *COMT Val*^{158}*Met* and *SLC6A4* 5-HTTLPR polymorphisms upon brain structure.

Conflict of Interest

None declared.

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