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Interaction of catechol *O*-methyltransferase and serotonin transporter genes modulates effective connectivity in a facial emotion-processing circuitry

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Imaging genetic studies showed exaggerated blood oxygenation level-dependent response in limbic structures in carriers of low activity alleles of serotonin transporter-linked promoter region (*5-HTTLPR*) as well as *catechol O-methyltransferase* (*COMT*) genes. This was suggested to underlie the vulnerability to mood disorders. To better understand the mechanisms of vulnerability, it is important to investigate the genetic modulation of frontal-limbic connectivity that underlies emotional regulation and control. In this study, we have examined the interaction of *5-HTTLPR* and *COMT* genetic markers on effective connectivity within neural circuitry for emotional facial expressions. A total of 91 healthy Caucasian adults underwent functional magnetic resonance imaging experiments with a task presenting dynamic emotional facial expressions of fear, sadness, happiness and anger. The effective connectivity within the facial processing circuitry was assessed with Granger causality method. We have demonstrated that in fear processing condition, an interaction between *5-HTTLPR (S)* and *COMT* (*met*) low activity alleles was associated with reduced reciprocal connectivity within the circuitry including bilateral fusiform/inferior occipital regions, right superior temporal gyrus/superior temporal sulcus, bilateral inferior/middle prefrontal cortex and right amygdala. We suggest that the epistatic effect of reduced effective connectivity may underlie an inefficient emotion regulation that places these individuals at greater risk for depressive disorders.
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Introduction

The last decade has seen the emergence of imaging genetics, as a research strategy to elucidate relationships between genotypic markers and neural structures or processes, that can help to identify pathophysiological processes predisposing to psychiatric disorders.[1] One consistent finding is exaggerated amygdala response to threat-related stimuli in carriers of low activity short (*S*) allele of the serotonin transporter-linked promoter region (*5-HTTLPR*) gene (see review[2]). This genetic modulation of brain response to emotionally negative signals has been highlighted as a potential mechanism underlying inefficiency of emotional processing and susceptibility to depressive disorders.[3] Another key genetic variation modulating emotional processes is the *val*158*met* single nucleotide polymorphism (SNP) of the gene coding for the catechol *O*-methyltransferase (*COMT*) enzyme that inactivates extraneuronal dopamine. Imaging genetic studies have reported that *met* carriers overactivated subcortical limbic regions in response to negative emotional stimuli.[4–6] These results suggest a role for the *met* allele in predisposing to greater stress reactivity[7] and a negative emotion attentional bias that may confer risk for affective disorders. Direct evidence for an association between *COMT* genotype and major depressive disorder (MDD) remains equivocal.[8] The investigators, however, emphasized the importance of gene–gene interaction (epistasis) in predisposing toward complex syndromes, such as MDD, that may occur even in the absence of main effects of single genes.[9,10] This is supported by the evidence of interactive effects of *COMT* and *5-HTTLPR* genotypes in predisposing toward development of MDD in individuals with a history of stressful life events.[11]

There is little research on interactive effects of the above genetic markers on neural response. To the best of our knowledge, the only imaging genetic study to examine the joint effect of *5-HTTLPR* and *COMT* genotypic variation upon neural activity in healthy individuals demonstrated additive effects of low activity *5-HTTLPR* and *COMT* alleles (*S* and *met* alleles, respectively), resulting in exaggerated limbic activity during the processing of emotionally unpleasant pictures.[12] This additive effect was suggested to underlie low resilience to dysphoric mood states in individuals carrying low activity alleles of both genes.

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As evidenced by recent studies, the measures of inter-regional brain connectivity may be more sensitive than the estimates of regional neural activity based on blood oxygenation level-dependent (BOLD) response.[13] The connectivity between amygdala and anterior prefrontal regions during the processing of negative emotional stimuli is proposed to underlie emotional regulation and control.[14,15] Importantly, the studies in depression found reduced connectivity between the limbic structures and anterior prefrontal regions, which was suggested to reflect inefficient emotion regulation.[16–19] This evidence is supporting a neural model of MDD[20] that emphasized a functional 'uncoupling' between anterior limbic and prefrontal cortical regions that are critically engaged during emotion regulation.

Findings regarding the impact of *COMT* and *5-HTTLPR* genotypic variations on functional connectivity in healthy individuals are, however, inconsistent. For example, the *5-HTTLPR S* allele has been associated with *greater* functional connectivity between the amygdala and rostral ventromedial prefrontal cortex[21] but also *decreased* functional connectivity between the amygdala and subgenual anterior cingulate cortex,[14] in response to emotionally negative visual stimuli. Similarly, *COMT met* allele carriers have shown *greater* functional connectivity between the right amygdala and ventrolateral prefrontal cortex[5] and right orbitofrontal cortex,[22] but also *reduced* effective connectivity between dorsolateral prefrontal cortex and ventral striatum.[23]

The aim of the present study was to examine the joint effect of *COMT* and *5-HTTLPR* functional polymorphisms upon the effective connectivity in neural circuitry, supporting facial emotion processing in healthy volunteers. We employed three novel approaches:

(1) We examined the interaction, as well as separate effects, of *COMT* and *5-HTTLPR* genetic variations upon emotion-processing neural circuitry.
(2) We focused on measures of effective connectivity employing Granger connectivity analysis.[24,25] This approach can identify the direction of informational flow between neural regions of interest unlike, for example, psychophysiological interaction, that focuses mainly on temporal relationships between the BOLD signal of regions of interest.[26] As a data-driven approach, Granger effective connectivity analysis also differs from structural equation modeling[27] and dynamic causal modeling,[28] that rely on *a-priori* network model specifications.[29,30]
(3) We employed a novel ecologically relevant paradigm that comprised dynamic facial emotional expressions. Previous studies have reported that neural regions supporting face emotion processing, including the amygdala and fusiform gyrus, were more strongly activated by dynamic vs static emotional faces.[31] Based on previous findings, we hypothesized that the carriers of low activity *COMT* and *5-HTTLPR* genotypes will demonstrate a pattern of inefficient emotion regulation represented by reduced Granger effective connectivity from prefrontal cortical to anterior limbic regions in response to threat-related facial emotions.

Methods

Participants. A total of 91 right-handed white Caucasian healthy individuals (45 female; age $= 32.5 \pm 9$, range 19–56 years) with no personal or family history of psychiatric disorder participated in four fMRI experiments. Exclusion criteria were current or past psychiatric diagnosis as established by the Structured Clinical Interview for DSM IV (SCID) screen.[32]

The study was approved by the Ethics Committee of the Institute of Psychiatry, King's College London, UK. Participants were provided with full details about the experimental protocol and gave their written informed consent before the beginning of the experiment.

Genotyping. The polymorphism *val*158*met* was considered for *COMT* genotyping, in line with the suggestions[33] based on direct comparisons of the effects of single *COMT val*158*met* SNP (rs4680) vs the haplotypes employed in previous studies.[34,35] It was demonstrated that the *val*158*met* polymorphism was more informative for understanding the effect of *COMT* on neural activity when compared with the haplotypes used by the above investigators.[34,35] We emphasize, however, that the findings of the comparison study[33] do not imply that haplotype-based models are generally inferior to models based on individual SNPs. In our case, the use of *COMT val*158*met* SNP was preferable in order to avoid unnecessary model complexity when applying an interaction analysis to the multiple connectivity data.

Regarding the *5-HTTLPR* gene, we considered both the well established difference between the higher expression *5-HTTLPR* long (*L*) allele vs the low expression *S* allele,[36] and the evidence regarding the impact of the rs25531 *G/A* SNP upon functioning of the *L* allele.[37] We reclassified *5-HTTLPR* alleles on the basis of lower and higher levels of expression similarly to previous reports.[38] Thus, *LG* and *S* were recoded as *S'*, whereas *LA* and *L* were recoded as *L'*.

DNA was extracted from cheek swabs using standard procedures (see Supplementary Method in the online supplement).

The *COMT met/met* homozygosity was detected in 29 participants and *val/val* in 20 participants; 41 participants were heterozygous. After recoding the *5-HTTLPR* alleles, as indicated above, 26 participants were homozygous *L'/L'*, 22 participants were homozygous *S'/S'* and 36 participants were heterozygous. There was no statistical deviation from Hardy–Weinberg equilibrium for all polymorphisms. The proportions of *COMT* and *5-HTTLPR* low activity genotypes were independent from each other (Spearman correlation between the number of *COMT met* alleles and the number of *5-HTTLPR S'* alleles: $\rho = -0.049$, $P = 0.647$). Due to low DNA yield, the data on *COMT* did not pass the quality control in one participant, and, on *5-HTTLPR*, in seven participants.

As the missing genotyping values represented only a small proportion ($<5\%$) of the whole data, to analyse the genetic effect on neural response and connectivity, we have employed the imputation approach, that is, genotyping data were filled in with the expectation maximization algorithm of the 'gc.em' procedure for R.[39]

Neuroimaging paradigm. There were four experimental runs, one per each of four facial expressions: fear, anger, sadness or happiness. The active condition stimuli were short monochromatic movie excerpts generated from the NimStim series of facial pictures (http://www.macbrain.org/), as described elsewhere.[40] In particular, in each excerpt, the facial expression changed from neutral to the emotional one over a period of 1 s. The models were males and females of Caucasian, African or Asian origin. Gender and racial distribution were similar in all emotional experiments. In each of the four emotional experimental runs, there were nine blocks of 12 movie clips per block. Each block lasted 42 s. Three of these blocks displayed facial expressions. The other blocks comprised either baseline (three blocks) or 'identity morph' (three blocks) conditions. The baseline blocks comprised presentation of 1 s movie excerpts containing monochromatic ovals. The ovals were approximately of the same size as that of the models' faces in the facial expression blocks. To control for the dynamic aspect of the facial expressions in active condition, each oval contained a smaller dynamic one with darker borders, concentrically expanding/moving from center to the perimeter. The third condition ('identity morphs') block contained 1 s movie excerpts in which one model with a non-emotional, neutral expression changed dynamically to another identity displaying a neutral expression. We did not include the analysis of identity morph blocks in the present study, as our focus was to compare activity and effective connectivity in facial expression relative to baseline blocks. To ensure that the participants were attending to the stimuli (in the second half of each movie excerpt a colored translucent filter (either orange, blue or yellow) appeared for 300 ms), the participants were requested to press the button with their right index finger as soon as they saw the color filter. To avoid habituation, the inter-stimulus interval between the movie clips was 'jittered', varying from 2000 to 2999 ms, mean = 2500 ms. Each experimental run (in which one of the four facial expression types was presented) lasted 6 min and 18 s. The participants completed all four experiments within the same session, with short breaks between them, during which they stayed in scanner, listening to the researcher reminding them the instructions through the intercom. The order of the emotional conditions was counterbalanced between participants.

Neuroimaging data acquisition. Scanning was performed on a GE Signa 3 Tesla scanner (Milwaukee, WI, USA). Reliable image quality was obtained by using a semi-automated quality control procedure. For BOLD imaging, 189 T2*-weighted whole-brain volumes were acquired during the experimental conditions. The EPI data set was acquired parallel to the intercommissural plane and consisted of 38 slices: TR = 2000 ms, TE = 25 ms, flip angle = 80°, slice thickness = 2.4 mm, inter-slice gap = 1.0 mm, image acquisition matrix size = 64^2. High-resolution structural images comprised 43 slices with slice thickness/gap 3.0/0.3 mm, TR = 3000 ms, TE = 30 ms, flip angle = 90 and matrix size = 128^2.

fMRI data analysis

a. The BOLD response to each emotional condition was analyzed with XBAM v4 (Institute of Psychiatry, London, UK), which is based on permutation testing that allows a mixed effects approach to analysis,[41] see also http://brainmap.it.
b. To identify the neural circuit responding to any of the four emotional conditions (general emotion-processing circuit), a binary map was produced where the BOLD responses to all four emotional expressions overlapped. This was necessary—in order to investigate the genetic effects on the same neural circuit across different emotional conditions.
c. Effective connectivity was examined based on Granger causality analysis that provides estimates of the *direction* of information flow between the nodes of the neural circuit. This information flow is estimated by the analysis of temporal precedence and could be either uni- or bi-directional (reciprocal). We have used cluster Granger analysis,[30] which is an extension of the original concept.[24,25] To deal with the high dimensionality of fMRI data, the cluster Granger analysis is employing principal component analysis, thus accounting for multiple time-series within each region of interest , rather than being related to the peak voxel or to the average time-series of the voxels within the region of interest. Cluster Granger analysis is using canonical correlations, which is also different from the recently introduced multivariate Granger analysis.[42]
d. To compare the strength in effective connectivity between individuals with different genotypes, we applied the measure of the 'total-degree'. This measure, in analogy to graph theory,[43] was defined as the total number of significant Granger-causalities in the general emotion-processing circuit. For instance, if Granger analyses in a given individual had detected 36 statistically significant Granger-causalities between the regions of interest, their total-degree was 36. When comparing genotypes, we looked at the statistical difference in total-degrees between the groups of individuals with different genetic polymorphisms.

Results

General emotion-processing circuit. Activation maps pertaining to processing of each of four emotional conditions showed the regions that were either emotion-general or relatively specific to each emotion (Table 1). The emotion-general map, where the clusters overlapped across all four conditions, included six neural regions: bilateral fusiform/inferior occipital regions (FOG, BA 19, 37, 18), right superior temporal gyrus/superior temporal sulcus (RSTG, BA 21, 22 and 39), bilateral inferior/middle prefrontal cortex (IFG, BA 9, 44, 45) and right amygdala (RAMG), Figure 1. The regions that were differentially activated in each condition, in addition to the general emotion-processing circuit, are shown in Supplementary Table S1.

Effects of genotypic variation in *5-HTTLPR* and *COMT* genes upon BOLD response in the general emotion-processing circuit. There were no significant effects of either *5-HTTLPR* or *COMT* genetic polymorphisms on BOLD signal in any of the six regions within the general emotion-

Table 1 Activation regions detected in different emotional conditions and the overlap regions

	Number of voxels	Maxima	
		Talairach x,y,z[a]	Corrected P
Fearful faces vs ovals[b]			
Bilateral temporo-occipito-cerebellar region BA 19, 37	545	32,−70,−16	0.0002
		−36,−70,−23	0.0002
Right MFG region, BA 6, 10	431	7,63,10	0.0002
Left IFG/middle frontal region, BA 6, 46	160	−43,33,13	0.0007
Right ventral striatum, amygdala	33	22,4,−13	0.001
Angry faces vs ovals[b]			
Bilateral temporo-occipito-cerebellar region BA 36	1000	36,−41,−20	0.0002
		−32,−67,−20	0.0002
Bilateral IFG/middle prefrontal region, BA 6, 9	388	40,15,26	0.0002
		−43,0,30	0.0005
Right ventral striatum, amygdala	48	18,4,−13	0.0005
Happy faces vs ovals[b]			
Bilateral temporo-occipito-cerebellar region, BA 19, 37	778	40,−67,−13	0.0003
		−40,−70,−20	0.0003
Bilateral middle frontal/precentral region, BA 9,46	624	43,30,13	0.0003
		−40,4,33	0.0003
Left STG/STS region, BA 22	51	−51,−44,3	0.001
Right parahippocampal region, amygdala	26	25,0,−13	0.001
Sad faces vs ovals[b]			
Bilateral IFG/precentral region, BA 6,45	1114	32,7,26	0.0002
		−36,26,7	0.0002
Bilateral temporo-occipito-cerebellar region	851	36,−41,−20	0.0002
		−36,−41,−23	0.0002
Right parahippocampal region, amygdala	47	22,0,−10	0.0007
Right STG/insula region, BA 13	37	36,−44,26	0.002
Overlap regions: general emotion-processing circuit			
Bilateral fusiform/occipital regions, BA 18, 19, 37	169	26,−60,−17	N/A
		−37,−64,−20	
Right STG/STS, BA 21,22	44	45,−41,7	N/A
Bilateral inferior/middle PFC, BA 9,44, 45	34	41,19,27	N/A
		−37,7,30	
Right parahippocampal region/amygdala	5	19,−4,−10	N/A

[a]Coordinates represent the voxel with maximum cluster activation in contrasts between emotional faces and ovals, and the voxel with minimum square distances to the other voxels of the cluster in overlap map.
[b]Contrasts between emotional faces and ovals were thresholded with voxel $P = 0.05$ and cluster $P = 0.01$.

processing circuit in any emotional condition. In the condition with fearful expressions only, the BOLD signal in RAMG was higher in low activity polymorphism carriers, although the difference did not reach significance: *COMT met/met > val/val*, $P = 0.13$ and *5-HTTLPR S'S' > L'L'*, $P = 0.26$.

Effective connectivity in the general emotion-processing circuit. In each emotional condition, all Granger connectivity pathways in the general emotion-processing circuit were statistically significant in both directions between neural regions (Supplementary Table S2), with no significant directionality difference except for one connection: in the fear condition the connectivity from the RSTG to right FOG was stronger than other way round (McNemar $\chi^2 = 10.3$, df = 1, FDR-corrected *P*-value = 0.038). However, in this link the effective connectivity in both directions remained highly significant. The effective connectivity in the entire general emotion-processing circuit was significant in each emotional condition (all $P \leqslant$ 3E-09), Figure 2.

Genetic effects upon effective connectivity in the general emotion-processing circuit. There was a significant negative relationship between the number of *S'* alleles of *5-HTTLPR* and total effective connectivity in the fearful condition only ($\rho = -0.26$, $P = 0.012$), but not in the conditions with sad, angry or happy faces. This *S'*-related progressive reduction of effective connectivity was seen in the contrasts between *L'/L'* and *L'/S'* genotypes (difference = 5.5; W = 792, $P = 0.004$), and between *L'/L'* and *S'/S'* genotypes (difference = 5.9; W = 399, $P = 0.020$).

Similarly, for the *COMT* gene, there was a significant negative correlation between the number of *met* alleles and total effective connectivity in the fearful condition only ($\rho = -0.27$, $P = 0.009$). There were significant differences between *val/val* vs *val/met* carriers (difference = 4.8; W = 567, $P = 0.027$) and *val/val* vs *met/met* carriers (difference = 6.2; W = 441, $P = 0.002$).

We next conducted an ANOVA with the following binary regressors: *COMT* (*val/val* vs either *val/met* or *met/met*),

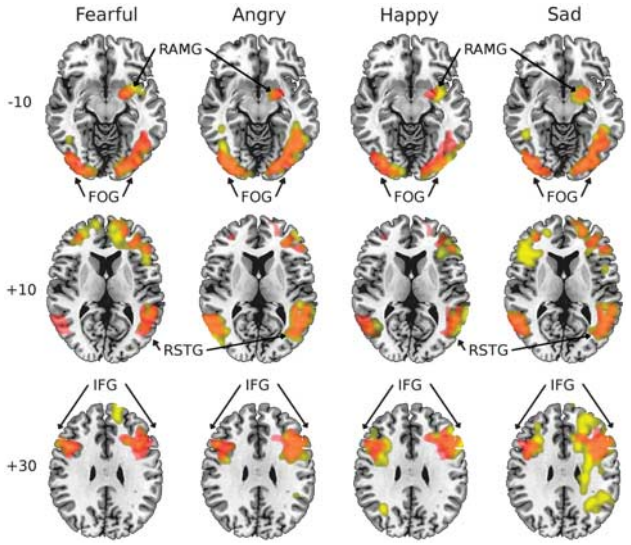

Figure 1 Activation regions detected in different emotional conditions. Axial slices at $z = -10$ (top), $+10$ (middle) and $+30$ (bottom) Showing brain regions with significant BOLD response to the facial emotional stimuli. The regions commonly activated by each emotional expression (general emotion-processing circuit) are shown in red. These include: bilateral fusiform/inferior occipital regions (FOG. BA 19; 37; 18), right superior temporal gyrus/superior temporal sulcus (RSTG. BA 21; 22 and 39) bilateral inferior/middle prefrontal cortex (IFG. BA 9; 44; 45) and right amygdala (RAMG). The regions that were additionally activated by particular emotional expressions are shown in yellow. Left side of the slice corresponds to the left side of the brain.

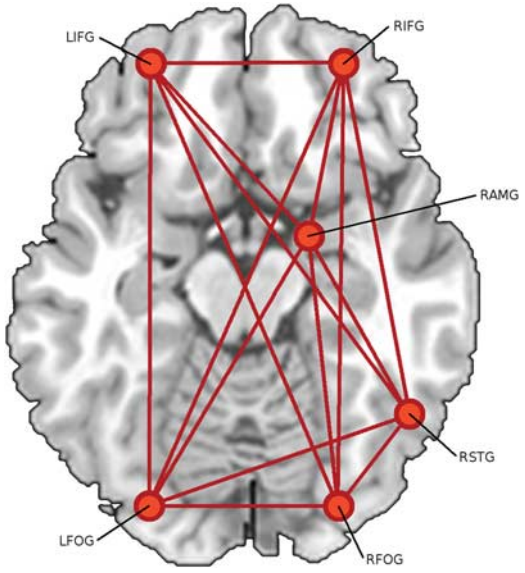

Figure 2 General emotion-processing circuit: fearful faces condition. Schematic depiction of inter-regional connections within the general emotion-processing circuit. Left side of the slice corresponds to the left side of the brain. LIFG, left inferior frontal gyrus; RIFG, right inferior frontal gyrus; RAMG, right amygdala; RSTG, right superior temporal gyrus; LFOG, left fusiform/occipital gyrus; RFOG, right fusiform/occipital gyrus.

5-HTTLPR (*L'/L'* vs either *L'/S'* or *S'/S'*) and *COMT* x *5-HTTLPR*, with total degree connectivity in fear condition as a dependent variable. The interaction model was

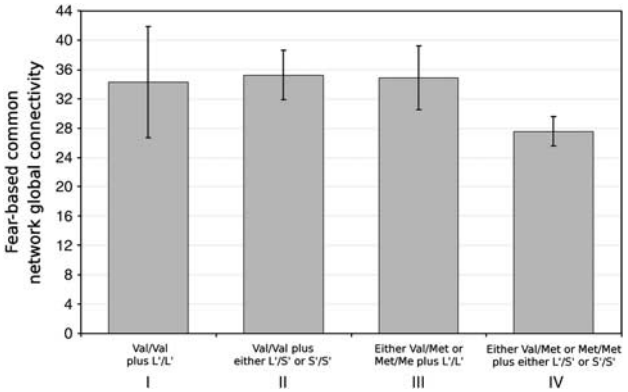

Figure 3 Effective connectivity in the general emotion-processing circuit: fearful faces condition. Bars represent the mean total-degree and error bars their 95% confidence intervals based on Student's *t*-distribution. The effective connectivity in individuals lacking at least one of the homozygotes *L'L'* or Val/Val (bar IV) is reduced, compared with those carrying any other combinations of alleles (bars I–III).

statistically significant ($F = 7.1$, df = 3,87, $P = 0.0003$, adjusted $R^2 = 17\%$). The interaction effect was stronger than an additive one ($F = 4.2$, df = 1, $P = 0.045$).

The effective connectivity in general emotion-processing circuit was lower only in *val/met* or *met/met* carriers who *at the same time* were either *L'/S'* or *S'/S'* carriers (mean total-degree = 27.6), compared with participants who were homozygous for either *val* or *L'* or were homozygous for both *val* and *L'* (mean total-degree = 34.9; difference = 7.3; W = 1575, $P = 7E\text{-}06$ (Figure 3).

Discussion

Our findings demonstrated significantly reduced reciprocal effective connectivity in the facial emotion-processing circuit in the carriers of low activity *5-HTTLPR S'* and *COMT met* alleles, relative to those with high activity homozygotes *5-HTTLPR L'L'* and *COMT val/val*. Furthermore, there was a significant interaction between *5-HTTLPR* and *COMT* polymorphisms upon effective connectivity in the face emotion-processing circuit. Effective connectivity was lower in *val/met* or *met/met* carriers who at the same time were either *L'/S'* or *S'/S'* carriers, compared with individuals who were homozygous for either *val* or *L'* alleles, or had both *val* and *L'* homozygotes. The above effects were observed in a fear condition, in the circuit comprising bilateral fusiform/occipital regions, bilateral inferior prefrontal cortex, right superior temporal gyrus/superior temporal sulcus and the right amygdala.

The combined effect of *5-HTTLPR* and *COMT* polymorphisms has been studied previously, albeit only with regard to the BOLD response to emotional pictures.[12] Our results concur, with the proposal of the above study,[12] that the joint effect of low activity *5-HTTLPR* and *COMT* polymorphisms confers inefficient emotion processing. The novelty of our findings is that the interactive effect of *5-HTTLPR* and *COMT* polymorphisms upon effective connectivity provides an insight into the genetically determined differences in emotion regulation, rather than emotion processing *per se*. We emphasize that above polymorphisms modulated

connectivity in a distributed neural circuit, rather than connectivity between *a-priori* selected prefrontal regions and amygdala. Importantly, although this circuit has been determined empirically, it is fully consistent with existing neural models for face processing.[44,45]

Our results are in line with the findings of decreased connectivity (uncoupling) between anterior prefrontal cortical regions and amygdala in healthy carriers of low activity alleles of *5-HTTLPR* in response to emotionally aversive stimuli.[14] Some previous studies have reported increased cortico–limbic connectivity in *5-HTTLPR S* allele carriers[21,46] and *COMT met/met* homozygotes.[5,22] We believe that our results do not contradict earlier results, given that it has previously been shown that connectivity between the brain regions depends on the precise prefrontal cortical regions examined. For example, one study[14] demonstrated both increased connectivity between ventro-medial prefrontal cortex and amygdala and decreased connectivity between subgenual ACC and amygdala in the *S* allele carriers vs *L* allele homozygotes of *5-HTTLPR*. We note here that the prefrontal cortical regions of the emotional circuit identified in our study were located dorsally and laterally to the ventro-medial regions of prefrontal cortex of other studies.[5,21,22,46] Our finding that low activity alleles of both *5-HTTLPR* and *COMT* genes (that is, alleles that are thought to confer risk for emotional disorders) are associated with reduced connectivity within a distributed face emotion-processing network are consistent with findings of reduced connectivity between prefrontal and ventral limbic regions in individuals with MDD,[16–19,29] or pathological anxiety,[47] including PTSD[48] and social anxiety disorder.[49]

What are the implications of the reduced connectivity within the emotion-processing circuit? The converging evidence from animal research,[50,51] neuroimaging data on healthy individuals[14,15,52] and the above studies in patients with depression and anxiety disorders indicate that reduced prefrontal-limbic connectivity may underlie inefficient emotion regulation during the processing of negative stimuli. We suggest that our findings of reduced connectivity within the emotion-processing neural circuit may be a necessary, although not sufficient component pathophysiological process in MDD. Indeed, the MDD is known to be associated with cellular and structural abnormalities in prefrontal[53] and temporal[54] cortices and/or abnormal reductions in prefrontal cortical activity.[55]

We briefly consider here the issue of directionality as examined with the Granger connectivity analysis. Neuroimaging genetic studies that have explored functional connectivity, found genetic modulation of connectivity between medial prefrontal and limbic regions.[14,21] This was suggested to indicate a modulation of top-down effect on emotion regulation. However, the assumption has not been formally tested, as functional connectivity is of correlational rather than a causal character. Using Granger effective connectivity, we specifically tested the potentially top-down relationships within the emotion-processing circuit.

Our data demonstrated that the frontal regions did not simply impact on limbic areas in a top-down manner, but there was a rather more complex functional organization that involved reciprocal feed-forward and feedback relationships. This included reduced *bi-directional* connectivity between the prefrontal cortex and amygdala in individuals with low activity *COMT* and 5HTTLPR alleles. We suggest that the finding of reduced bi-directionality does not contradict the notion of reduced top-down emotion regulation in low activity carriers as it includes both top-down and bottom-up mechanisms. Thus, our data add a new aspect (that is, feedback mechanisms) to the frontal-limbic relationships.

Therefore, our results are in an agreement with the above studies that were based on correlational methods.

In contrast to the above studies that were testing a coupling between the two *a-priori* defined regions, we were able to study effective connectivity within the facial emotion-processing circuit that was established empirically. Our findings add to the existing knowledge by showing that the observed reduced connectivity is not just a correlational in nature but involves both top-down and feedback projections.

We emphasize that finding of bi-directional relationship within cortico–limbic network is in agreement with existing experimental literature,[56–58] supporting 'longitudinal' rather than a rigidly hierarchical network models. It has been shown[59] that cortico–striato–pallido–thalamo–cortical circuitry was arranged as a series of circuits (closed loops).

The recent studies based on Granger connectivity have also demonstrated resting state[60,61] or task-dependent[62] uni- and bidirectional effective connectivity.

Another important issue that deserves consideration is the emotion-specificity of the genetic effect.

In our study, all emotional expressions, that is, angry, sad, fearful and happy faces activated emotion-processing circuit; however, the genetic effect on connectivity within this circuit was observed in the fearful condition only. As both angry and fearful facial expressions represent threat-related cues, it is important to consider the possible reasons for lack of the genetic effect in angry faces condition.

Although angry faces clearly provide information about the presence of threat, it has been found that the fearful facial expressions (as signaling more ambiguous threat) have been consistently associated with the strongest amygdala activation. This is in accord with the conceptualization of amygdala as a component of vigilance system responding to ambiguous situations of biological relevance.[63] This was evident in a study combining fMRI with skin conductance recording,[64] where the fearful (but not angry) faces elicited activation in amygdala-dependent arousal system for fight/flight, which is recognizable fear network. Given a crucial role of serotonin in modulation of the brain processes underlying responses to potential environmental threats, it is conceivable that the processing of fearful faces would be modulated by the serotonin neurotransmission. Indeed, the processing of fearful but not angry faces has been consistently associated with the serotonin metabolism (see review).[65] In support of our results, the recognition of fearful but not angry faces was modulated by the *5-HTTLPR S vs L*.[66] There is little evidence of a differential effect of dopaminergic transmission on angry vs fearful faces processing. The investigators report the *COMT* effect on fear processing in general, for example, the *COMT met* homozygosity was associated with the lack of ability to extinguish conditioned fear, whereas *5-HTTLPR S* homozygosity underlied a potentiation of startle reactions.[67]

The authors concluded that that the combination of a *5-HTTLPR S* allele and *COMT met*-homozygosity conferred an enhanced risk for acquiring fear that resisted extinction.

These data support our findings of a gene–gene effect of *COMT* and *5-HTTLPR* genotypes on fear processing.

Limitations. The absence of an effect of low activity *5-HTTLPR* and *COMT* polymorphisms on BOLD signal in limbic regions needs consideration. The effect of *5-HTTLPR* on BOLD signal in the amygdala has been replicated in most of the studies,[2] although not all of them had sufficient power—as indicated in a meta-analysis.[68] The effect of *COMT val/met* polymorphisms on amygdala activation has not been consistently replicated, for example, it was reported by some[4] but not other investigators.[5] The authors highlighted an importance of baseline stimuli that was not controlled for in some previous studies. Thus, an exaggerated BOLD signal in amygdala to emotional vs neutral stimuli could have been accounted for by significantly greater amygdala response to the baseline, that is, fixation cross, perceived as an ambiguous signal by *S*-allele carriers.[69,70] However, a recent study that directly tested the effect of a fixation cross on BOLD response[71] did not replicate the findings of greater activation to fixation cross relative to the neutral faces.

Dynamic facial stimuli used in our study represent relatively new type of experimental stimuli. Although they provide for closest possible analogy to the socially occurring events, developing an adequate baseline condition proved to be a challenging task. There is little knowledge regarding the perceptual effect of the baseline condition—moving ovals—which may come across as emotionally ambiguous signals. This may have resulted in a greater amygdala activity in *S*- and *met*-allele carriers such that the net BOLD signal changes to emotional faces vs baseline condition was not significantly exaggerated in these individuals. Thus, this should be tested in further research. Alternatively, the absence of a genetic effect on BOLD response in amygdala should not be regarded as a false negative, but rather genuine, statistically plausible negative result. Indeed, a proportion of studies with negative results is expected even if there is a true relationship between *COMT* (or *5-HTTLPR*) and the BOLD response in amygdala to aversive signals. For instance if the statistical power of neuroimaging genetic studies was as high as 90%, still, 1 out of 10 studies should not detect the effect. Therefore, we suggest that the negative results have to be reported in order to avoid the publication bias, which distorts the real state of neuroimaging research.

We emphasize that the main focus of this study was on effective connectivity within the emotion-processing circuit, the measure of which (Granger causality) is based on temporal precedence and thus is not directly related to magnitude of the BOLD effect.

As mentioned above, due to some missing data, we have used the imputation method. To exclude any false positive results due to the imputation, we re-analyzed the data using the data only pertaining to the 84 individuals with full genotyping information. The interaction remained significant: $F = 6.4$, $df = 3.79$; $P = 0.0006$, adjusted $R^2 = 16\%$.

Conclusion

Our results indicate that the interaction of *5-HTTLPR* and *COMT* low activity alleles may be associated with reduced *reciprocal* connectivity within the emotion-processing circuit that includes frontal, temporal, occipital regions and right amygdala. This epistatic effect may underlie an inefficient emotion regulation in these individuals that increases the risk to MDD.

Conflict of interest

The authors declare no conflict of interest.

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