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Resting-state cerebral blood flow in amygdala is modulated by sex and serotonin transporter genotype

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ABSTRACT

Serotonin transporter-linked polymorphic region (5-HTTLPR) has been associated with modulation of resting-state amygdala level, which was considered to underlie a risk for mood and anxiety disorders. The findings however have been inconsistent which could be related to interactions of the genotype with other factors e.g. sex or personality characteristics. Therefore, the aim of the present study was to explore the modulation of the amygdala perfusion in the resting-state by sex and 5-HTTLPR/rs25531 genotype, controlled for personality dimensions assessed by Temperament and Character Inventory (Cloninger et al., 1994). The resting-state cerebral blood flow (rCBF) was examined using an arterial spin labelling technique. All participants were genotyped for the 5-HTTLPR/rs25531 genotype (*L/L–L/S–S/S* genotypes and L_A–L_G variants). The study group comprised 81 right-handed Caucasian healthy volunteers (42 females) aged 19–55 years. We measured rCBF in the amygdala and in the whole-brain grey matter. The data of blood-oxygen-level-dependent (BOLD) response in amygdala to fearful dynamic faces in the same sample were also analysed. There was a significant main effect of sex in both the left and right amygdalae, with higher rCBF in males. Main effect of 5-HTTLPR/rs25531 genotype which was significant in the right amygdala only, was accounted for by higher rCBF in *S/S* vs. *L/L* homozygotes. An interaction between sex and 5-HTTLPR/rs25531 genotype was observed in rCBF in the right amygdala. This was accounted for by higher values of rCBF in the right amygdala in males' *S* allele carriers compared with females. In females, there was a significant negative correlation between the rCBF and BOLD response in the right amygdala, and more so in *S* carriers. In males, there was no significant correlation between rCBF and BOLD response in the right amygdala. The novelty of our results lies in the demonstration of gene by sex interaction with resting blood flow in the amygdala that elucidates sex-related differences in emotional reactivity.

Introduction

Amygdala functioning has been implicated in emotional reactivity (Olsson and Phelps, 2007; Whalen, 1998) and various forms of emotional psychopathology (Anand and Shekhar, 2003; Surguladze et al., 2010). Patients suffering from affective disorders often show excessive amygdala response to aversive signals and reduced prefrontal cortex functionality, which tends to normalise after pharmacotherapy using selective serotonin reuptake inhibitors (Quidé et al., 2012). Previous imaging genetic studies looking for intermediate phenotypes for affective disorders suggested that serotonin transporter gene (SLC6A4) variation modulated amygdala response to aversive signals. Initially, the serotonin-transporter-linked polymorphic region (5-HTTLPR) of the SLC6A4 gene was characterised by two alleles of different functionalities with regard to uptake of serotonin. In particular, the short (*S*) allele is associated with reduced transcription efficiency for the gene, resulting in decreased expression of the serotonin transporter compared with the long *L* allele (Lesch et al., 1996). Hu et al. (2006) showed that 5-HTTLPR is functionally triallelic (*S*, L_A, and L_G alleles); the L_G and *S* alleles are low-expressing whilst the L_A allele is high-expressing. Neuroimaging studies employing blood-oxygen-level-dependent (BOLD) response – task-related rather than resting-state activity – demonstrated increased amygdala activation in *S* allele carriers as compared to the homozygous long *L* allele carriers (Hariri et al., 2002, 2005; Munafo et al., 2008;

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Scharinger et al., 2010). The increased amygdala reactivity in *S* allele carriers has been suggested to underpin susceptibility to affective disorders.

Recent research demonstrated possibilities of examining resting-state amygdala modulation by 5-HTTLPR. In a pioneering multimodal study, using both BOLD response imaging and CASL (Continuous Arterial Spin Labelling), Canli et al. (2006) reported an increased amygdala resting-state cerebral blood flow (rCBF) in *S* carriers with high rates of life stress. This demonstration of gene × environment interaction effect on rCBF in amygdala was of particular importance given the evidence that 5-HTTLPR and stress interact to predict predisposition to depression (Caspi et al., 2003; Kendler et al., 2005). Subsequent study (Rao et al., 2007) replicated the results of Canli et al. (2006) demonstrating significantly increased rCBF in the bilateral amygdala in *S/S* homozygotes and decreased rCBF in the ventromedial prefrontal cortex, compared with the *L/L* homozygotes. Canli et al. (2005) argued that resting-state (or tonic) amygdala activity, rather than task-related (phasic) amygdala response, may determine emotional reactivity and risk of affective disorders. The converging evidence from the resting state Positron Emission Tomography (PET) studies also provide support for the above proposal by showing an abnormally increased amygdala metabolism in depression (Abercrombie et al., 1998; Drevets, 2003; Drevets et al., 1992). Recent demonstration of increased resting state perfusion in amygdala of individuals with major depression, points to the same direction (Brockmann et al., 2011).

However, the results of other studies do not conform to Canli's proposal (Canli et al., 2005, 2006) of elevated tonic activity in amygdala in *S* carriers. For example, an fMRI study (von dem Hagen et al., 2011) that specifically examined various baseline conditions, found increased amygdala BOLD response to emotional rather than baseline stimuli in *S* carriers. This supports the 'phasic' rather than 'tonic' activation hypothesis. The largest study to date employing CASL, Viviani et al. (2010) found no 5-HTTLPR effect on resting rCBF in the amygdala which again did not support the hypothesis of tonic hyperactivity in risk allele carriers.

There could be various reasons for the above inconsistencies. E.g., the studies of Canli et al. (2006) and Rao et al. (2007) have examined traditional S/L variants of 5-HTTLPR polymorphisms, whereas Viviani et al. (2010) looked at the effect of S allele and the *Lg* and *La* variants of the long repeat. The inconsistent results could also be due to interactions of the genotype with other factors, such as stress (Alexander et al., 2012; El-Hage et al., 2009) or sex (He et al., 2010). Importantly, Viviani et al. (2010) demonstrated a significant effect of sex on regional cerebral blood flow (rCBF) in amygdala with lower rCBF in females. There is evidence of sex-dependent differences in the amygdala structure and function (for review see (Hamann, 2005). E.g., in males compared with females, amygdala has been found to have larger volume (Goldstein et al., 2001), increased metabolism (Gur et al., 1995) and higher values of rCBF (Viviani et al., 2010). It has been suggested that the sex differences in limbic (particularly, amygdala) function may underlie differential susceptibility to mood disorders (Davidson et al., 2002). This is in line with the behavioural experiments (Burton et al., 2005; Collignon et al., 2010), including our recent study (Gohier et al., 2013), in which healthy females have consistently demonstrated increased sensitivity to negative emotional signals. Most importantly for the studies of serotonergic gene effect on brain activity, a sex effect on serotonergic function has also been reported — e.g. investigators have demonstrated that serotonin has different (even opposite) effects on behavioural characteristics in males versus females (Brummett et al., 2008). Thus, it seems imperative to take into account the effect of sex when studying the effect of serotonin transporter gene on amygdala rCBF.

An additional factor that might affect the baseline rCBF is the personality profile. Some studies showed the significant association between the amygdala activity, personality dimensions and the modulatory effect of the serotonin transporter gene (e.g., Bertolino et al., 2005; Hariri et al., 2005; Munafo et al., 2008; Surguladze et al., 2008). However, all of these studies collected BOLD fMRI data. Recently, Li et al. (2012) showed sex-based differences in the association between harm avoidance scores and amygdala resting-state functional connectivity to a number of brain regions. O'Gorman et al. (2006) have also reported the associations between rCBF in limbic structures and dimensions of personality as assessed by Eysenck Personality Questionnaire (Eysenck and Eysenck, 1991) and Temperament and Character Inventory (TCI) (Cloninger et al., 1994). Importantly, the subjects of the study (Rao et al., 2007) that reported an effect of 5-HTTLPR on rCBF in amygdala have been matched in terms of personality and mood measures.

Taking into consideration the above evidence, the current study examined an interactive effect of 5-HTTLPR and sex on resting-state amygdala activity in healthy individuals using pulsed-Continuous Arterial Spin Labelling (pCASL) (Dai et al., 2008). The influence of the personality dimensions on rCBF was controlled using the TCI (Cloninger et al., 1994). BOLD-fMRI data were collected for comparison with the pCASL results. Since sex differences in amygdala functioning have been found during emotional tasks (Domes et al., 2010; Wrase et al., 2003), in terms of glucose metabolism (Gur et al., 1995), and rCBF (Viviani et al., 2010), we sought to replicate the finding of a sex effect on rCBF in the amygdala with higher amygdala activity in males compared to females. Based on sex-related differences in serotonergic function, we also expected that the effect of 5-HTTLPR gene on rCBF in amygdala will be different in males and females.

Methods

Participants

We recruited 97 subjects aged between 18 and 55 years. Exclusion criteria were current or past substance abuse, head injury, and/or any pre-existing neuropsychiatric disorder, screened out by the Structured Clinical Interview for DSM-IV-TR (Diagnostic and Statistical Manual of Mental Disorders) axis 1 disorders (First et al., 2001). Seven participants were excluded for various reasons (e.g. brain tumour detected by MRI, claustrophobia, and missing data). Moreover, two subjects were excluded from the study due to problems in perfusion quantification and seven participants due to low DNA yield. The final sample included 81 healthy right-handed Caucasian volunteers (42 females) aged 19–55 years (32.3 ± 8.9). The study was conducted in accordance with the Institutional Ethics Review Board of the Institute of Psychiatry, London, UK. Participants were given full details about the experimental protocol and gave their written informed consent before the beginning of the experiments.

All participants were screened with the mini-mental state examination (MMSE) (Folstein et al., 1975), General Health Questionnaire (GHQ) (Goldberg and Hillier, 1979) and the National Adult Reading Test (NART) (Nelson, 1982). Mood and anxiety characteristics were assessed with Beck Depression Inventory (BDI) (Beck et al., 1986) and State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1970) respectively. The dimensions of personality were examined with TCI (Cloninger et al., 1994).

DNA extraction and genotyping

Each participant provided cheek swab samples and DNA was extracted using standard protocols developed at the Social, Genetic and Developmental Psychiatry Centre (Freeman et al., 2003). We considered both the well-established difference between the higher expression 5-HTTLPR long (*L*) allele vs. the low expression short (*S*) allele (Heils et al., 1996) and the evidence regarding the impact of the rs25531 *G/A* Single Nucleotide Polymorphism (SNP) upon functioning of the *L* allele (triallelic distribution) (Hu et al., 2006), in an attempt to replicate the study of Viviani et al. (2010). We reclassified 5-HTTLPR alleles on the basis of lower and higher levels of expression similarly