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Epistasis between 5-HTTLPR and ADRA2B polymorphisms influences attentional bias for emotional information in healthy volunteers

Kris H. Naudts¹, Ruben T. Azevedo², Anthony S. David², Kees van Heeringen³
and Ayana A. Gibbs^{2,4}

¹ Department of Forensic and Neurodevelopmental Sciences, Institute of Psychiatry, King's College London, King's Health Partners, London, UK

² Department of Psychosis Studies, Institute of Psychiatry, King's College London, King's Health Partners, London, UK

³ Department of Psychiatry and Medical Psychology, Ghent University, Ghent, Belgium

⁴ Division of Clinical Medicine, Brighton and Sussex Medical School, Falmer, Brighton, UK

Abstract

Individual differences in emotional processing are likely to contribute to vulnerability and resilience to emotional disorders such as depression and anxiety. Genetic variation is known to contribute to these differences but they remain incompletely understood. The serotonin transporter (5-HTTLPR) and α_2B -adrenergic autoreceptor (ADRA2B) insertion/deletion polymorphisms impact on two separate but interacting monoaminergic signalling mechanisms that have been implicated in both emotional processing and emotional disorders. Recent studies suggest that the 5-HTTLPR *s* allele is associated with a negative attentional bias and an increased risk of emotional disorders. However, such complex behavioural traits are likely to exhibit polygenicity, including epistasis. This study examined the contribution of the 5-HTTLPR and ADRA2B insertion/deletion polymorphisms to attentional biases for aversive information in 94 healthy male volunteers and found evidence of a significant epistatic effect ($p < 0.001$). Specifically, in the presence of the 5-HTTLPR *s* allele, the attentional bias for aversive information was attenuated by possession of the ADRA2B deletion variant whereas in the absence of the *s* allele, the bias was enhanced. These data identify a cognitive mechanism linking genotype-dependent serotonergic and noradrenergic signalling that is likely to have implications for the development of cognitive markers for depression/anxiety as well as therapeutic drug effects and personalized approaches to treatment.

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Introduction

Enhanced processing of emotionally salient information in relation to neutral information is normally considered to be an adaptive process enabling threat detection and increasing the probability of survival (Vuilleumier, 2005). However, there is considerable evidence that biased processing of emotional information also plays a role in the aetiology and maintenance of emotional disorders such as depression and anxiety (Leppanen, 2006). The monoamine neurotransmitters (serotonin, dopamine, noradrenaline) are known to play a

significant role in emotional processing and although they are generally considered to act synergistically, few studies have specifically investigated interactions between these neurotransmitters.

Serotonin and noradrenaline are also heavily implicated in the aetiology of emotional disorders and the majority of therapeutic agents increase synaptic levels of these neurotransmitters (Nutt, 2002). Despite their use for over half a century, it remains unclear how antidepressants exert their therapeutic effects. More recently, evidence has emerged suggesting that serotonergic and noradrenergic antidepressant drugs may act by modifying emotional processing biases (Harmer *et al.* 2009). Yet in spite of this increasing insight, the fact that up to 50% of patients treated with these medications fail to respond adequately remains

Address for correspondence: Dr A. A. Gibbs, Clinical Imaging Sciences Centre, University of Sussex, Falmer, Brighton BN1 9RR, UK.
Tel.: 01273876702 Fax: 01273877548
Email: a.gibbs@bsms.ac.uk

a significant challenge in the management of these disorders (Souery *et al.* 1999). Inter-individual differences in responses to emotional stimuli may contribute to differences in vulnerability to emotional disorders as well as response to therapeutic agents (Hamann & Canli, 2004). It is increasingly accepted that genetic factors explain small but significant amounts of this variability (Todd *et al.* 2011). Thus, polymorphisms in genes involved in serotonergic and noradrenergic signalling represent apposite candidates for further investigation.

One of the genetic variants that have been most extensively investigated in relation to human emotional processing and emotional disorders is the gene encoding the serotonin transporter (5-HTT or SLC6A4). An insertion/deletion polymorphism in the promoter region of this gene (5-HTTLPR) results in two common allelic variants: short (*s*) and long (*l*). The former has been associated with reduced transporter transcription, resulting in approximately 50% reduction in transporter availability *in vitro* and presumed increased synaptic serotonin availability (Heils *et al.* 1996). More recently, an additional A/G single nucleotide polymorphism (SNP) in the *l* allele (rs25531) has been found to further influence transcriptional activity. The G variant of the *l* allele is considered to result in a reduction in transcriptional efficiency to a level similar to that of the *s* allele (Hu *et al.* 2005; Wendland *et al.* 2006). However, the frequency of this G allele varies with ethnicity and it is relatively uncommon in white European ethnic groups (Hu *et al.* 2006). Early seminal studies linked the *s* allele to increased neurotic personality traits (Lesch *et al.* 1996) and an increased risk of depression in the context of adverse life events (Caspi *et al.* 2003). Subsequent studies examining the *in-vivo* effects of this genetic variation on the phenotypic expression of 5-HTT in the human brain have produced inconsistent results (Praschak-Rieder *et al.* 2007; Reimold *et al.* 2007; van Dyck *et al.* 2004; Willeit *et al.* 2000). However, consistent with a number of previous studies, a relatively large recent positron emission tomography (PET) study in healthy volunteers found that polymorphic variation in 5-HTTLPR did not alter expression of 5-HTT (Murthy *et al.* 2010). It has, however, been suggested that this genetic variation instead contributes to early neurodevelopmental changes that may impact on brain structure and function in later life (Lesch & Gutknecht, 2005). This would be consistent with the further body of functional magnetic resonance imaging (fMRI) literature that has more consistently documented that *s* allele carriers demonstrate significantly greater amygdala activation in response to

aversive, relative to neutral, stimuli in a variety of emotional processing tasks (Bertolino *et al.* 2005; Canli *et al.* 2005; Hariri *et al.* 2002, 2005; Heinz *et al.* 2004; Pezawas *et al.* 2005; for a meta-analysis see Munafò *et al.* 2008). Yet, the behavioural implications of these neural differences remained unclear. More recently a number of studies have focused on 'behavioural endophenotypes' such as selective attentional biases for emotional information. To date, these studies have demonstrated an association between the 5-HTTLPR *s* allele and preferential attention to aversive stimuli (Beavers *et al.* 2007, 2010, 2011; Fox *et al.* 2009; Osinsky *et al.* 2008), although these reports have not been entirely consistent (Caspi *et al.* 2010). One important potential source of inconsistency and non-replication in genetics studies of complex quantitative traits is the issue of polygenicity, including biological epistasis (Moore, 2008). However, none of these neuroimaging or behavioural genetics studies has examined the effects of the other major neurotransmitter system implicated in affective spectrum disorders and emotional processing, i.e. the noradrenergic system.

Noradrenaline has an established role in modulating memory enhancement for emotionally arousing information (McGaugh, 2004) and recent pharmacological challenge studies indicate that it is also involved in modulating attentional biases for emotional information in healthy human volunteers (De Martino *et al.* 2008). However, the contribution of genetically influenced differences in noradrenergic tone to inter-individual differences in human emotional processing has been largely unexplored. An insertion/deletion polymorphism in the α_{2B} -adrenergic (auto)receptor gene (ADRA2B) has recently been found to contribute to individual differences in emotionally influenced memory processes. The deletion variant (Del301-303) is associated with decreased agonist-promoted phosphorylation and receptor desensitization *in vitro* (Small *et al.* 2001), presumed to be associated with increased noradrenergic tone *in vivo*. In two seminal studies, de Quervain and colleagues demonstrated an association between this polymorphic variant, increased amygdala reactivity and an increased memory bias for emotional stimuli (de Quervain *et al.* 2007; Rasch *et al.* 2009). However, the contribution of ADRA2B to emotionally enhanced attentional processes was not explored. It therefore remains possible that the observed memory bias arises due to an attentional advantage contributing to enhanced encoding of emotional information (Todd *et al.* 2011). The purpose of this study was therefore to test the hypothesis that an increased attentional bias for emotionally arousing information is associated with the deletion variants of

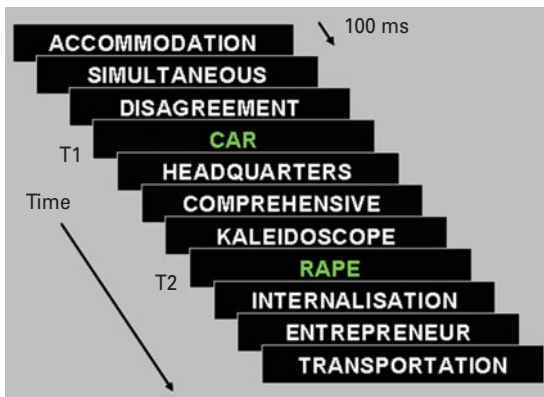


Fig. 1. Schematic representation of attentional blink task.

ADRA2B and 5-HTTLPR and examine whether these effects are subject to additive or non-additive interactions.

Materials and methods

Participants

One hundred and seven healthy white British male volunteers between the ages of 18 and 35 yr (mean \pm s.d. = 24.0 \pm 4.8) were recruited from the university and local community. They had no lifetime history of psychiatric or neurological disorder. Estimates of verbal IQ were derived from the National Adult Reading Test (NART; Nelson, 1982). The study was approved by the local research ethics committee. Following complete description of the study to the participants, written informed consent was obtained.

Behavioural task

We used an emotional attention blink (AB) task based on dual-target rapid serial visual presentation (RSVP) methodology (Raymond *et al.* 1992). Identification of a first target (T1) in a rapid stream of stimuli leads to transient impairment in identification of a second target (T2) – an effect, known as the *attentional blink*. It has previously been used by us and others to demonstrate a bias towards accurate detection of aversive T2 targets compared to neutral (Anderson, 2005; Anderson & Phelps, 2001; De Martino *et al.* 2008; Gibbs *et al.* 2007; Keil & Ihssen, 2004). The task comprised 168 trials, each trial consisting of 13 white distracter words and 2 green target words (T1 and T2) presented sequentially in the centre of a laptop computer screen (see Fig. 1). T1 stimuli were all neutral words averaging 4.8 letters in length. T2 words were derived from the Affective Norms for English Words (Bradley & Lawson, 1999) and half

were aversive-arousing (mean valence and arousal ratings of 2.5 and 7.0, respectively) and half were neutral (mean valence and arousal ratings of 5.1 and 3.5, respectively). Aversive and neutral T2 stimuli did not differ significantly in letter length (mean = 5.1 *vs.* 4.8, respectively, $p = 0.21$) or written word frequency (mean = 67.1 *vs.* 87.9, respectively, $p = 0.50$) (Kucera & Francis, 1967). Distracter items were 92 words of longer length (mean letters = 12.5) to facilitate masking of the targets. Each item was presented for 100 ms and was immediately followed by the subsequent item. The lag between the T1 and T2 targets was varied to contain one, three, or five intervening distracters (lag 2, lag 4 or lag 6) with corresponding stimulus onset asynchronies (SOAs) of 200 ms, 400 ms or 600 ms. Participants were instructed to ignore the words in white (distracters) and identify the two green target words (T1 and T2). Responses were made by participants writing down the two targets in any order immediately after each trial on sheets that were subsequently scored. Exact correct spelling was not necessary for a correct response. Vowel and consonant omissions, insertions or replacements were allowed provided the word was recognizable and the spelling was phonologically accurate.

Genotyping

DNA extraction and genotyping for the ADRA2B insertion/deletion polymorphism was performed by KBioscience, Hertfordshire, UK as previously reported (Gibbs *et al.* 2010). For the 5-HTTLPR insertion/deletion, polymerase chain reaction (PCR) was also performed using KBioscience's in-house SNP genotyping system (KASPar[®]) using fluorescently labelled primers (pF1: Cy5.5-CCCAGCGTGCTCCAGAAAC; pR: GGACCTGGGCAGTTGTGC). For technical reasons, we were unable to complete further tri-allelic genotyping of the A/G SNP (rs25531) in the 5-HTTLPR insertion allele.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) of genetic data was assessed by χ^2 analysis. Possible genotype-dependent differences in demographic variables between genotype groups were assessed in a multivariate analysis of variance (ANOVA) with age and IQ as dependent variables and 5-HTTLPR and ADRA2B genotypes as between-subjects factors. Genotype effects on T1 detection in the AB task were examined in a univariate ANOVA with percent correct T1 report as the dependent measure and 5-HTTLPR and ADRA2B genotypes as the between-subjects factors. Affective

Table 1. Demographic characteristics for 5-HTTLPR and ADRA2B genotypes

	5-HTTLPR				ADRA2B			
	S	L	F	<i>p</i>	Del	Ins	F	<i>p</i>
Number of participants	34	60	–	–	51	43	–	–
Age, yr mean (s.d.)	23.9 (4.9)	23.9 (4.3)	0.02 (–)	0.88 (–)	24.7 (4.9)	23.0 (4.3)	1.18 (–)	0.31 (–)
IQ mean (s.d.)	105.9 (6.5)	104.4 (7.0)	2.28 (–)	0.14 (–)	106.7 (5.7)	104.7 (6.4)	3.01 (–)	0.06 (–)

modulation of the AB effect was examined in a repeated-measures ANOVA with the same between-subjects factors, valence (aversive, neutral) and lag (2, 4, 6) as within-subject factors and percent correct T2 report (contingent on the correct identification of T1)[†] as the dependent measure. Significant interactions were explored using *post-hoc t* tests. A Greenhouse–Geisser correction was applied where sphericity assumptions were violated.

Results

Genotypes

Of the 107 participants, ADRA2B genotypes were unavailable for two participants, 11 were homozygous carriers of the ADRA2B deletion, 48 were heterozygotes and 46 were non-carriers, consistent with HWE ($\chi^2=0.09$, $p=0.77$). Due to the small number of homozygous carriers, they were combined with the heterozygotes, giving two genotype groups of deletion carriers (Del) and non-carriers (Ins) as previously done by us and others (de Quervain *et al.* 2007; Gibbs *et al.* 2010). For 5-HTTLPR, 12 genotypes were unavailable, 35 were homozygous *l/l*, 41 were heterozygous *s/l* and 19 were homozygous *s/s*, consistent with HWE ($\chi^2=1.18$, $p=0.28$). Given that the *s* allele is considered to have a dominant effect (Lesch *et al.* 1996), participants were divided into two groups: homozygous or heterozygous *s* allele carriers (S group) and non-carriers (L group) consistent with previous studies (Canli *et al.* 2005). Participants for whom genetic data were not available for both polymorphisms were excluded from further analysis, leaving a total sample of 94. Demographic characteristics (age and IQ) are given in Table 1. There were no genotype effects on these variables.

Behavioural data

There was a significant main effect of valence on T2 detection accuracy [$F(1, 90)=11.0$, $p=0.001$], with greater detection of aversive ($92.7 \pm 1.6\%$) compared to neutral ($81.2 \pm 1.2\%$) words. T2 detection accuracy also increased significantly [$F(1.2, 109.4)=183.3$, $p<0.001$] as the temporal lag between the targets increased [lag 2 = $64.4 \pm 22.2\%$; lag 4 = $83.8 \pm 15.7\%$; lag 6 = $91.1 \pm 12.3\%$] and there was a significant lag \times valence interaction [$F(2, 180)=28.5$, $p<0.001$] such that the emotional attentional bias was most pronounced at lag 4 (9%) compared to lag 6 (3%) and lag 2 (–3%) where it was absent. There were no main effects of the individual genes; however, there was a highly significant ADRA2B \times 5-HTTLPR \times valence interaction [$F(1, 90)=15.0$, $p<0.001$]. In order to clarify this interaction we first conducted a separate repeated-measures ANOVA in the 5-HTTLPR S and L groups with T2 detection as the dependent variable, valence (aversive, neutral) as the between-subjects variable and ADRA2B genotype as the between-subjects variable. We found significant ADRA2B \times valence interactions in both 5-HTTLPR S [$F(1, 58)=8.2$, $p=0.006$] and L [$F(1, 32)=6.06$, $p=0.02$] groups. *Post-hoc* paired *t* tests demonstrated that in the 5-HTTLPR L group, there was a significant attentional bias for aversive *vs.* neutral T2 words in ADRA2B deletion carriers [$t(20)=3.0$, $p=0.007$, $d=0.7$] that was absent in non-carriers [$t(12)=-0.68$, $p=0.504$]. Conversely, in the 5-HTTLPR S group, the significant emotional attentional bias was absent in ADRA2B deletion carriers [$t(29)=0.477$, $p=0.637$] but present in non-carriers [$t(29)=4.9$, $p<0.001$, $d=1$] (see Fig. 2). There were no other significant main genotype effects or interactions in relation to T1 or T2 detection.

Discussion

The purpose of this study was to examine the effects of serotonergic (5-HTTLPR) and noradrenergic (ADRA2B) genetic variants on attentional biases for aversive stimuli using an attentional blink (AB)

[†] This is to guarantee that proper attention has been devoted to T1 to ensure an AB effect.

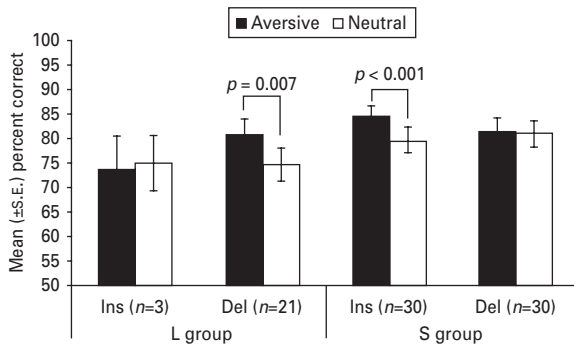


Fig. 2. Accuracy (percent correct) of T2 detection in 5-HTTLPR and ADRA2B groups.

paradigm. To our knowledge, this is the first study to examine the contribution of the ADRA2B insertion/deletion polymorphism to individual differences in emotional attentional biases and only the second study to explore the genetic basis of the emotional AB effect [Munafò and colleagues previously found an association between 5-HTTLPR genotype, smoking status and detection of smoking-related stimuli in an AB task (Munafò *et al.* 2005)]. The significant novel finding from this study is that the affective modulation of T2 detection is influenced by a non-additive (epistatic) interaction between the ADRA2B and 5-HTTLPR insertion/deletion polymorphisms.

Specifically, we found that a significant attentional bias for aversive compared to neutral information was present in individuals possessing at least one copy of the short (*s*) allele of 5-HTTLPR, but only if they did not carry the ADRA2B deletion. Conversely, the attentional bias for emotional information was only present in 5-HTTLPR long (*l*) allele homozygotes if they were ADRA2B deletion carriers. This suggests that in the presence of the 5-HTTLPR *s* allele which is a putative risk allele for depressive and anxiety disorders, the negative attentional bias is attenuated by the ADRA2B deletion variant whereas in the absence of the *s* allele, the bias is enhanced. Both of these effects may be related to adaptive processes. For instance, dependent on 5-HTTLPR genotype, the effect of the ADRA2B deletion variant may be to either exert a protective effect against affective spectrum disorders or facilitate enhanced detection of threat, in both cases contributing to increased probability of survival.

Behavioural genetics implications

We did not find a main effect of 5-HTTLPR on emotional attention as a number of previous studies have done (Beevers *et al.* 2007; Fox *et al.* 2009; Munafò *et al.* 2005; Osinsky *et al.* 2008). However, with the

exception of Munafò *et al.* (2005), all of these studies used a variation of the dot probe task to evaluate emotional biases in selective attention, rather than the AB task. Although both of these tasks evaluate selective attention when cognitive resources are limited, the latter measures deployment of attention resources under temporal constraints while the former typically utilizes spatial limitations. Notably, Munafò *et al.* (2005) also used an alternate variant of the AB task that indexes attention by establishing whether the detection of a neutral T2 target is impaired when preceded by an emotionally salient or neutral T1 target. It is possible that these task-related differences may account for the difference in findings. Yet the studies reporting positive associations using the dot probe task are not without inconsistencies. For example some have linked the *s* allele to biases towards aversive stimuli (Beevers *et al.* 2007; Osinsky *et al.* 2008) while others suggest that the *l* allele results in biases away from negative stimuli (Fox *et al.* 2009; Kwang *et al.* 2010). Other discrepancies include 5-HTTLPR associations found only with long (Osinsky *et al.* 2008) or short (Beevers *et al.* 2007) stimulus presentation durations. In spite of using shared dot probe methodology, there are still significant differences between these studies in terms of subjects (healthy volunteers *vs.* psychiatric patients; men *vs.* women), stimuli (words *vs.* spiders *vs.* pictorial scenes) and duration of stimulus presentation (<500 ms *vs.* >500 ms). These differences highlight the need for task consistency in future studies in order to facilitate replication (NCI-NHGRI Working Group on Replication in Association Studies, 2007).

The effect of the ADRA2B insertion/deletion polymorphism on attentional biases for emotional information has not been previously investigated. However, it has been suggested that it might contribute to the emotional memory bias observed in ADRA2B deletion carriers (Todd & Anderson, 2009). We did not find any main effect of ADRA2B on emotional attention in this study suggesting that the ADRA2B deletion variant does not independently bias attention towards emotional stimuli but may interact with other monoaminergic gene systems to contribute to such bias.

Behavioural pharmacogenetics implications

A number of studies have begun to examine possible interactions between 5-HTTLPR polymorphisms, emotional attentional biases and the effects of serotonergic manipulation by acute tryptophan depletion (ATD) but have thus far failed to produce consistent

findings (Firk & Markus, 2009; Markus & De Raedt, 2011; Markus & Firk, 2009; Roiser et al. 2007). This may in part be due to the fact that ATD in healthy volunteers, independent of genotype, has not produced entirely consistent effects on emotional processing (Hayward et al. 2005; Murphy et al. 2002; Rubinsztein et al. 2001). No studies have as yet examined the possible contribution of genetic variation to the effects of serotonergic and noradrenergic drugs on emotional attention. However, a number of pharmacological studies have examined the effects of serotonergic and noradrenergic drugs on emotional processing in healthy subjects (Arce et al. 2008; Arnone et al. 2009; Brühl et al. 2009; Harmer et al. 2008, 2003, 2004; Murphy et al. 2009a; Norbury et al. 2007; Rawlings et al. 2010). This work may be relevant to understanding the present findings; however, it is difficult to make direct comparisons between genetically and pharmacologically mediated effects on emotional processing as highlighted in the Clinical implications subsection below. Additionally, only three of these studies have specifically examined attentional biases and these have produced relatively inconsistent findings (Browning et al. 2007; De Martino et al. 2008; Murphy et al. 2009b).

Using a dot probe task, Browning et al. (2007) found that the administration of a single dose of the selective serotonin reuptake inhibitor (SSRI) antidepressant citalopram to healthy volunteers resulted in an attentional bias towards positive words (Browning et al. 2007). This is consistent with some of the behavioural findings in relation to the 5-HTTLPR *l* allele described above. On an AB task, De Martino and colleagues found that a single dose of the noradrenaline reuptake inhibitor (NRI) reboxetine boosted detection of emotionally arousing compared to neutral words in healthy volunteers (De Martino et al. 2008). Also on a dot probe task in healthy volunteers, Murphy et al. (2009a,b) found that repeated citalopram administration reduced the attentional bias towards emotional faces, independently of valence, while reboxetine had no effect (Murphy et al. 2009b). These apparently conflicting findings could again be related to differences in methodology (dot probe vs. AB, words vs. faces, single vs. repeated dosing). In fact, there is evidence from both animal and human studies that acute and chronic citalopram administration may differentially influence emotional processing such that acute doses result in an initial increase in the processing of negative information that is attenuated with repeated dosing (Burghardt et al. 2004; Harmer et al. 2003, 2004). However genotype-dependent drug effects may also contribute to these differences. For

example, pharmacological enhancement or attenuation of emotional attentional biases may be less prominent in individuals with genotype-related emotional processing biases or in whom such biases are absent, respectively. If emotional attentional bias is to function as an effective cognitive marker, future pharmacological challenge studies will also need to consider the contribution of genetic variations in the neurotransmitter systems under investigation. There is also an increasing need to evaluate the effects of genetic epistasis between these systems as this may have important clinical implications for the pharmacogenetics of depression and anxiety.

Clinical implications

That such biological epistasis exists between serotonergic and noradrenergic genes, is consistent with the fact that these neurotransmitter systems are intimately connected in the central nervous system (de Boer, 1995). Noradrenergic neurotransmission is modulated by presynaptic inhibitory α_2 -adrenergic (auto)receptors and their blockade increases synaptic levels of noradrenaline. However, there is evidence that serotonergic neurotransmission is also modulated by presynaptic α_2 -adrenergic (hetero)receptors (Clement et al. 1992; De Boer et al. 1994; Mongeau et al. 1993). Yet precisely how these systems may interact to produce the intermediate phenotypes and the clinical disorders themselves remains unclear. The fact that the majority of drugs used to treat affective spectrum disorders act by inhibiting the serotonin transporter seems at odds with the fact that individuals with genetically influenced reductions in serotonin transporter function have greater risks of developing these disorders, as well as poorer treatment response rates (Lesch & Gutknecht, 2005). This ostensible contradiction is increasingly understood in terms of the complex autoregulatory processes governing serotonergic function (Routledge & Middlemiss, 1996) and the potentially deleterious neurodevelopmental effects of excessive intra-synaptic accumulation of serotonin (Lesch & Gutknecht, 2005). Via its intimate relationship with serotonergic signalling, the ADRA2B polymorphism may also exert its epistatic effects via these autoregulatory and neurodevelopmental mechanisms.

While this hypothesis warrants further investigation, the biological epistasis suggested in the present study may have important implications for individual responses to serotonergic and noradrenergic antidepressant drugs. Most pharmacogenetics studies in depression have focused on variations in 5-HTT (Schosser & Kasper, 2009; Serretti et al. 2007).

However, two large recent projects (STAR*D and GENDEP) have found associations between antidepressant response and a number of candidate genes involved in both serotonin and noradrenaline signalling (Hu *et al.* 2007; McMahon *et al.* 2006; Paddock *et al.* 2007; Uher *et al.* 2009), although none of these studies included the ADRA2B polymorphism. The GENDEP project did examine a polymorphism in the related ADRA2A gene encoding α_{2A} -adrenoceptor subtype but failed to find any significant effect despite a previously reported association with the response to the serotonin-noradrenaline reuptake inhibitor milnacipran (Wakeno *et al.* 2008). Of note, neither of the two antidepressants evaluated in GENDEP was a molecular target of the α_2 -adrenoceptor group.

Thus, the role of polymorphic variation in α_2 -adrenergic receptors (and their interaction with serotonergic targets) in the therapeutic response of patients with affective spectrum disorders warrants further investigation.

Study limitations

The purpose of this study was to investigate epistatic effects of serotonergic and noradrenergic genes on emotional attentional biases. However, the fact that we measured only two polymorphisms out of a number that might contribute to the behavioural effect of interest represents a limitation to this study. Most significantly, we were unable to genotype the additional rs25531 SNP in the long allele of the 5-HTT gene. However, given that the prevalence of the L_G allele is low (~10%), this is unlikely to have significantly biased our findings. A further limitation is that although the overall sample size was reasonable, there were relatively few individuals in some genotype combinations. This is a particular difficulty inherent in measuring epistatic gene effects (Moore, 2008). Future studies will need to use large sample sizes and evolving methodologies to effectively evaluate the likely effects on emotional processing of multiple gene interactions (Cordell, 2009). The final limitation is that we only used aversive stimuli and male volunteers. While the latter eliminated possible biases associated with gender differences in emotional processing, it limits the generalizability of our findings. It is therefore unclear whether the observed biases are valence and/or gender specific. Additionally the aversive stimuli used included range of negative emotions (disgust, fear, sadness) rather than specifically dysphoric or threat-related emotions. It is therefore unclear how these processing biases might map onto those considered to relate to depression and anxiety

disorders. Further studies with larger sample sizes including men and women will be required to replicate and extend our findings.

Conclusions

In spite of these limitations, this study begins to contribute to the understanding of multiple gene effects and interactions in an established cognitive marker for affective spectrum disorders – the negative attentional bias. It further underlines the potential utility of adopting the ‘endophenotype’ approach in pharmacogenetics studies, i.e. examining the genetic factors underlying not only the clinical response but also responses in cognitive and neural markers. One significant challenge for such studies will be to delineate the interaction between potential neurodevelopmental effects of genetic polymorphisms influencing brain neurotransmitter systems and the acute/sub-acute effects of drug administration. Knockout and transgenic mouse models are likely to be useful in appreciating the dynamics of the behavioural–psychopharmacogenetic–neurodevelopmental interface.

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Statement of Interest

None.

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