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

Article (Accepted Version)

CAMBRIDGE JOURNALS

Proof Delivery Form

International Journal of Neuropsychopharmacology

Date of delivery:

Journal and vol/article ref: pnp 0_0/1100129

Number of pages (not including this page): 10

This proof is sent to you on behalf of Cambridge University Press.

To expedite online publication of your paper, please email corrections within 2 days of receipt to:

sjparr@ntlworld.com

You are responsible for correcting your proof. Errors not found may appear in the published journal.

The proof is sent for typographical correction only. Revision of the substance of the text is not permitted, unless agreed with the Editor of the journal.

A new version of a figure must be supplied electronically if corrections are substantial. Minor corrections can be done by the typesetters. If in any doubt contact: kstanford@cambridge.org

Author queries:

AQ1: Supply telephone & fax details.
AQ2: Serotonin transporter (5-HTT) has been defined at first mention and abbreviation used thereafter.
AQ3: Text amended to ''mean $\pm$ S.D.'' – OK?
AQ4: Change ''the serotonin transporter polymorphism'' to ''5-HTTLPR''?
AQ5: Supply volume number for Moore (2008).
AQ6: Supply full publication details for Schosser & Kasper (2009).
Note to Author: Papers are generally published online within 4 weeks of receipt of the corrected proof.

Offprint order form

CAMBRIDGE UNIVERSITY PRESS

PLEASE COMPLETE AND RETURN THIS FORM. WE WILL BE UNABLE TO SEND OFFPRINTS (INCLUDING FREE OFFPRINTS) UNLESS A RETURN ADDRESS AND ARTICLE DETAILS ARE PROVIDED.

VAT REG NO. GB 823 8476 09

International Journal of Neuropsychopharmacology (PNP)

Volume: ☐ *no:* ☐

Offprints

Authors will receive a PDF file of the final version of their article. To also order offprints, please complete this form and send it to **the publisher (address below)**. Please give the address to which your offprints should be sent. They will be despatched by surface mail within one month of publication. For an article by **more than one author this form is sent to you as the first named. All extra offprints should be ordered by you in consultation with your co-authors.**

Number of offprints required: ☐

Email: .....

Offprints to be sent to (print in BLOCK CAPITALS): .....

.....

.....

.....

Post/Zip Code: .....

Telephone: ..... *Date (dd/mm/yy):* ..... / ..... / .....

Author(s): .....

.....

Article Title: .....

.....

*All enquiries about offprints should be addressed to **the publisher**: Kathy Stanford, Journals Production Department, Cambridge University Press, The Edinburgh Building, Shaftesbury Road, Cambridge CB2 2RU, UK.*

Charges for extra offprints (excluding VAT) *Please circle the appropriate charge:*

Number of copies	25	50	100	150	200	per 50 extra
1-4 pages	£68	£109	£174	£239	£309	£68
5-8 pages	£109	£163	£239	£321	£399	£109
9-16 pages	£120	£181	£285	£381	£494	£120
17-24 pages	£131	£201	£331	£451	£599	£131
Each Additional 1-8 pages	£20	£31	£50	£70	£104	£20

Methods of payment

If you live in Belgium, France, Germany, Ireland, Italy, Portugal, Spain or Sweden and are not registered for VAT we are required to charge VAT at the rate applicable in your country of residence. If you live in any other country in the EU and are not registered for VAT you will be charged VAT at the UK rate. If registered, please quote your VAT number, or the VAT number of any agency paying on your behalf if it is registered. *VAT Number:* .....

Payment **must** be included with your order, please tick which method you are using:

☐ Cheques should be made out to Cambridge University Press.

☐ Payment by someone else. Please enclose the official order when returning this form and ensure that when the order is sent it mentions the name of the journal and the article title.

☐ Payment may be made by any credit card bearing the Interbank Symbol.

Card Number: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Expiry Date (mm/yy): ..... / ..... *Card Verification Number:* ☐☐☐☐

The card verification number is a 3 digit number printed on the **back** of your **Visa** or **Master card**, it appears after and to the right of your card number. For **American Express** the verification number is 4 digits, and printed on the **front** of your card, after and to the right of your card number.

Signature of card holder: .....

Amount (Including VAT if appropriate): £.....

Please advise if address registered with card company is different from above

CAMBRIDGE UNIVERSITY PRESS

Transfer of copyright

Please read the notes overleaf and then complete, sign, and return this form to **Journals Production, Cambridge University Press, University Printing House, Shaftesbury Road, Cambridge, CB2 8BS, UK** as soon as possible.

In consideration of the publication in **INTERNATIONAL JOURNAL OF NEUROPSYCHOPHARMACOLOGY**

of the contribution entitled: .....

.....

by (all authors' names): .....

.....

1 To be filled in if copyright belongs to you

Transfer of copyright

I/we hereby assign to the Collegium Internationale Neuro-Psychopharmacologicum, full copyright in all formats and media in the said contribution, including in any supplementary materials that I/we may author in support of the online version.

I/we warrant that I am/we are the sole owner or co-owners of the contribution and have full power to make this agreement, and that the contribution contains nothing that is in any way an infringement of any existing copyright or licence, or duty of confidentiality, or duty to respect privacy, or any other right of any person or party whatsoever and contains nothing libellous or unlawful; and that all statements purporting to be facts are true and that any recipe, formula, instruction or equivalent published in the Journal will not, if followed accurately, cause any injury or damage to the user.

I/we further warrant that permission has been obtained from the copyright holder for any material not in my/our copyright including any audio and video material, that the appropriate acknowledgement has been made to the original source, and that in the case of audio or video material appropriate releases have been obtained from persons whose voices or likenesses are represented therein. I/we attach copies of all permission and release correspondence.

I/we hereby assert my/our moral rights in accordance with the UK Copyrights Designs and Patents Act (1988).

Signed (tick one)

☐ the sole author(s) ☐one author authorised to execute this transfer on behalf of all the authors of the above article

Name (block letters) .....

Institution/Company .....

Signature: ..... Date: .....

(Additional authors should provide this information on a separate sheet.)

2 To be filled in if copyright does not belong to you

a Name and address of copyright holder .....

.....

.....

b The copyright holder hereby grants to Collegium Internationale Neuro-Psychopharmacologicum the non-exclusive right to publish the contribution in the journal and to deal with requests from third parties in the manner specified in paragraphs 4 and 5 overleaf.

(Signature of copyright holder or authorised agent) .....

3 US Government exemption

I/we certify that the paper above was written in the course of employment by the United States Government so that no copyright exists.

Signature: ..... Name (Block letters): .....

4 Requests received by Cambridge University Press for permission to reprint this article (see para. 4 overleaf) should be sent to

Name and address (block letters) .....

Notes for contributors

1 The Journal's policy is to acquire copyright in all contributions. There are two reasons for this: *(a)* ownership of copyright by one central organisation tends to ensure maximum international protection against unauthorised use; *(b)* it also ensures that requests by third parties to reprint or reproduce a contribution, or part of it, are handled efficiently and in accordance with a general policy that is sensitive both to any relevant changes in international copyright legislation and to the general desirability of encouraging the dissemination of knowledge.

2 Two 'moral rights' were conferred on authors by the UK Copyright Act in 1988. In the UK an author's 'right of paternity', the right to be properly credited whenever the work is published (or performed or broadcast), requires that this right is asserted in writing.

3 Notwithstanding the assignment of copyright in their contribution, all contributors retain the following **non-transferable** rights:

- The right to post *either* their own version of their contribution as submitted to the journal (prior to revision arising from peer review and prior to editorial input by Cambridge University Press) *or* their own final version of their contribution as accepted for publication (subsequent to revision arising from peer review but still prior to editorial input by Cambridge University Press) on their **personal or departmental web page**, or in the **Institutional Repository** of the institution in which they worked at the time the paper was first submitted, or (for appropriate journals) in PubMedCentral or UK PubMedCentral, provided the posting is accompanied by a prominent statement that the paper has been accepted for publication and will appear in a revised form, subsequent to peer review and/or editorial input by Cambridge University Press, in **International Journal of Neuropsychopharmacology** published by Cambridge University Press, together with a copyright notice in the name of the copyright holder (Cambridge University Press or the sponsoring Society, as appropriate). On publication the full bibliographical details of the paper (volume: issue number (date), page numbers) must be inserted after the journal title, along with a link to the Cambridge website address for the journal. Inclusion of this version of the paper in Institutional Repositories outside of the institution in which the contributor worked at the time the paper was first submitted will be subject to the additional permission of Cambridge University Press (not to be unreasonably withheld).
- The right to post the definitive version of the contribution as published at Cambridge Journals Online (in PDF or HTML form) on their **personal or departmental web page**, no sooner than upon its appearance at Cambridge Journals Online, subject to file availability and provided the posting includes a prominent statement of the full bibliographical details, a copyright notice in the name of the copyright holder (Cambridge University Press or the sponsoring Society, as appropriate), and a link to the online edition of the journal at Cambridge Journals Online.
- The right to post the definitive version of the contribution as published at Cambridge Journals Online (in PDF or HTML form) in the **Institutional Repository** of the institution in which they worked at the time the paper was first submitted, or (for appropriate journals) in PubMedCentral or UK PubMedCentral, no sooner than **one year** after first publication of the paper in the journal, subject to file availability and provided the posting includes a prominent statement of the full bibliographical details, a copyright notice in the name of the copyright holder (Cambridge University Press or the sponsoring Society, as appropriate), and a link to the online edition of the journal at Cambridge Journals Online. Inclusion of this definitive version after one year in Institutional Repositories outside of the institution in which the contributor worked at the time the paper was first submitted will be subject to the additional permission of Cambridge University Press (not to be unreasonably withheld).
- The right to post an abstract of the contribution (for appropriate journals) on the **Social Science Research Network (SSRN)**, provided the abstract is accompanied by a prominent statement that the full contribution appears in **International Journal of Neuropsychopharmacology** published by Cambridge University Press, together with full bibliographical details, a copyright notice in the name of the journal's copyright holder (Cambridge University Press or the sponsoring Society, as appropriate), and a link to the online edition of the journal at Cambridge Journals Online.
- The right to make hard copies of the contribution or an adapted version for their own purposes, including the right to make multiple copies for course use by their students, provided no sale is involved.
- The right to reproduce the paper or an adapted version of it in any volume of which they are editor or author. Permission will automatically be given to the publisher of such a volume, subject to normal acknowledgement.

4 We shall use our best endeavours to ensure that any direct request we receive to reproduce your contribution, or a substantial part of it, in another publication (which may be an electronic publication) is approved by you before permission is given.

5 Cambridge University Press co-operates in various licensing schemes that allow material to be photocopied within agreed restraints (e.g. the CCC in the USA and the CLA in the UK). Any proceeds received from such licences, together with any proceeds from sales of subsidiary rights in the Journal, directly support its continuing publication.

6 It is understood that in some cases copyright will be held by the contributor's employer. If so, the Collegium Internationale Neuro-Psychopharmacologicum requires non-exclusive permission to deal with requests from third parties, on the understanding that any requests it receives from third parties will be handled in accordance with paragraphs 4 and 5 above (note that your approval and not that of your employer will be sought for the proposed use).

7 Permission to include material not in your copyright
If your contribution includes textual or illustrative material not in your copyright and not covered by fair use / fair dealing, permission must be obtained from the relevant copyright owner (usually the publisher or via the publisher) for the non-exclusive right to reproduce the material worldwide in all forms and media, including electronic publication. The relevant permission correspondence should be attached to this form.

If you are in doubt about whether or not permission is required, please consult the Permissions Manager, Cambridge University Press, The Edinburgh Building, Shaftesbury Road, Cambridge CB2 8RU, UK. Fax: +44 (0)1223 315052. Email: lnicol@cambridge.org.

The information provided on this form will be held in perpetuity for record purposes. The name(s) and address(es) of the author(s) of the contribution may be reproduced in the journal and provided to print and online indexing and abstracting services and bibliographic databases.

Please make a duplicate of this form for your own records

International Journal of Neuropsychopharmacology, Page 1 of 10. © CINP 2011
doi:10.1017/S1461145711001295

ARTICLE

CINP

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

Kris H. Naudts[1], Ruben T. Azevedo[2], Anthony S. David[2], Kees van Heeringen[3] 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] *Department of Psychiatry, 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 monaminergic 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.

Received 10 June 2011; Reviewed 7 July 2011; Revised 14 July 2011; Accepted 20 July 2011

Key words: ADRA2B, emotional processing, 5-HTTLPR.

Introduction

Enhanced processing of emotionally salient 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

AQ1 Address for correspondence: Dr A. A. Gibbs, Institute of Psychiatry, De Crespigny Park, PO Box 68, London SE19 3SS, UK.
Tel.: ■ *Fax*: ■
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 (Beevers *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

COLOUR

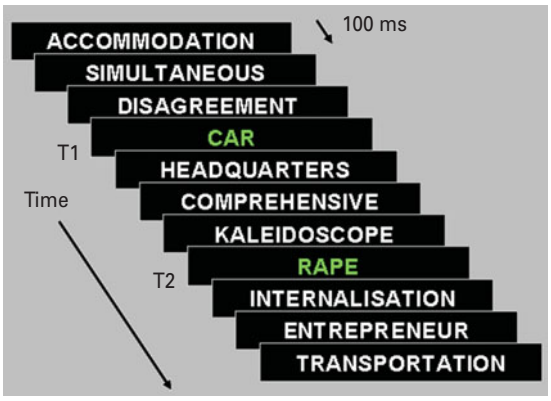

Fig. 1. Schematic representation of attentional blink task.

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 ± S.D. = 24.0 ± 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*	*p*	Del	Ins	*F*	*p*
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.

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

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 × 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 × 5-HTTLPR × 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 × valence interactions in both 5-HTLPR 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)

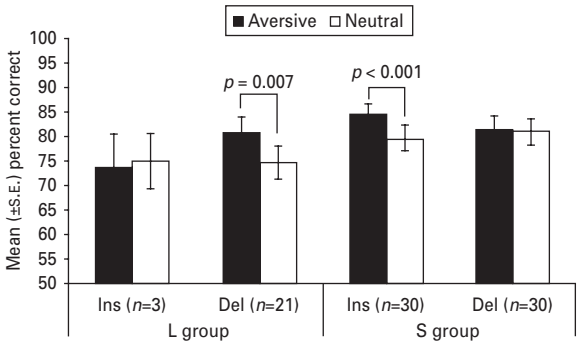

Fig. 2. Accuracy (percent correct) of T2 detection in 5-HTTLPR and ADRA2B groups.

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 the serotonin transporter polymorphism 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 constrains 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 preceding 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 monaminergic 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.* 2009*a*; 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.* 2009*b*).

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.* (2009*a*, *b*) found that repeated citalopram administration reduced the attentional bias towards emotional faces, independently of valence, while reboxetine had no effect (Murphy *et al.* 2009*b*). 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 5-HTT seems at odds with the fact that individuals with genetically influenced reductions in 5-HTT 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 ($\sim$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.

Acknowledgements

This study was funded by a pilot funding award from the NIHR Biomedical Research Centre for Mental Health at the South London and Maudsley NHS Foundation Trust and (Institute of Psychiatry) King's College London.

Statement of Interest

None.

References

Anderson AK (2005). Affective influences on the attentional dynamics supporting awareness. *Journal of Experimental Psychology: General* **134**, 258–281.

Anderson AK, Phelps EA (2001). Lesions of the human amygdala impair enhanced perception of emotionally salient events. *Nature* **411**, 305–309.

Arce E, Simmons A, Lovero K, Stein M, *et al.* (2008). Escitalopram effects on insula and amygdala BOLD activation during emotional processing. *Psychopharmacology* **196**, 661–672.

Arnone D, Horder J, Cowen P, Harmer C (2009). Early effects of mirtazapine on emotional processing. *Psychopharmacology* **203**, 685–691.

Beevers CG, Ellis AJ, Wells TT, McGeary JE (2010). Serotonin transporter gene promoter region

polymorphism and selective processing of emotional images. *Biological Psychology* **83**, 260–265.

Beevers CG, Gibb BE, McGeary JE, Miller IW (2007). Serotonin transporter genetic variation and biased attention for emotional word stimuli among psychiatric inpatients. *Journal of Abnormal Psychology* **116**, 208–212.

Beevers CG, Marti CN, Lee HJ, Stote DL, *et al.* (2011). Associations between serotonin transporter gene promoter region (5-HTTLPR) polymorphism and gaze bias for emotional information. *Journal of Abnormal Psychology* **120**, 187–197.

Bertolino A, Arciero G, Rubino V, Latorre V, *et al.* (2005). Variation of human amygdala response during threatening stimuli as a function of 5′HTTLPR genotype and personality style. *Biological Psychiatry* **57**, 1517–1525.

Bradley MM, Lawson ML (1999). *Affective Norms for English Words* (*ANEW*). Gainsville, FL: NIMH Center for the Study of Emotion and Attention.

Browning M, Reid C, Cowen PJ, Goodwin GM, *et al.* (2007). A single dose of citalopram increases fear recognition in healthy subjects. *Journal of Psychopharmacology* **21**, 684–690.

Brühl AB, Kaffenberger T, Herwig U (2009). Serotonergic and noradrenergic modulation of emotion processing by single dose antidepressants. *Neuropsychopharmacology* **35**, 521–533.

Burghardt NS, Sullivan GM, McEwen BS, Gorman JM, *et al.* (2004). The selective serotonin reuptake inhibitor citalopram increases fear after acute treatment but reduces fear with chronic treatment: a comparison with tianeptine. *Biological Psychiatry* **55**, 1171–1178.

Canli T, Omura K, Haas B, Fallgatter A, *et al.* (2005). Beyond affect: a role for genetic variation of the serotonin transporter in neural activation during a cognitive attention task. *Proceedings of the National Academy of Sciences USA* **102**, 12224–12229.

Caspi A, Hariri AR, Holmes A, Uher R, *et al.* (2010). Genetic sensitivity to the environment: the case of the serotonin transporter gene and its implications for studying complex diseases and traits. *American Journal of Psychiatry* **167**, 509–527.

Caspi A, Sugden K, Moffitt TE, Taylor A, *et al.* (2003). Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* **301**, 386–389.

Clement HW, Gemsa D, Wesemann W (1992). The effect of adrenergic drugs on serotonin metabolism in the nucleus raphe dorsalis of the rat, studied by *in vivo* voltammetry. *European Journal of Pharmacology* **217**, 43–48.

Cordell HJ (2009). Detecting gene–gene interactions that underlie human diseases. *Nature Reviews Genetics* **10**, 392–404.

de Boer T (1995). The effects of mirtazapine on central noradrenergic and serotonergic neurotransmission. *International Clinical Psychopharmacology* **10** (Suppl. 4), 19–23.

De Boer T, Nefkens F, Van Helvoirt A (1994). The α_2-adrenoceptor antagonist org 3770 enhances serotonin transmission *in vivo*. *European Journal of Pharmacology* **253**, R5–R6.

De Martino B, Strange B, Dolan R (2008). Noradrenergic neuromodulation of human attention for emotional and neutral stimuli. *Psychopharmacology* **197**, 127–136.

de Quervain DJF, Kolassa IT, Ertl V, Onyut PL, *et al.* (2007). A deletion variant of the α2b-adrenoceptor is related to emotional memory in Europeans and Africans. *Nature Neuroscience* **10**, 1137–1139.

Firk C, Markus C (2009). Differential effects of 5-HTTLPR genotypes on mood, memory, and attention bias following acute tryptophan depletion and stress exposure. *Psychopharmacology* **203**, 805–818.

Fox E, Ridgewell A, Ashwin C (2009). Looking on the bright side: biased attention and the human serotonin transporter gene. *Proceedings of the Royal Society of London, Series B: Biological Sciences* **276**, 1747–1751.

Gibbs A, Naudts K, Spencer E, David A (2007). The role of dopamine in attentional and memory biases for emotional information. *American Journal of Psychiatry* **164**, 1603–1609.

Gibbs AA, Naudts KH, Azevedo RT, David AS (2010). Deletion variant of α2b-adrenergic receptor gene moderates the effect of COMT val158met polymorphism on episodic memory performance. *European Neuropsychopharmacology* **20**, 272–275.

Hamann S, Canli T (2004). Individual differences in emotion processing. *Current Opinion in Neurobiology* **14**, 233–238.

Hariri AR, Drabant EM, Munoz KE, Kolachana BS, *et al.* (2005). A susceptibility gene for affective disorders and the response of the human amygdala. *Archives of General Psychiatry* **62**, 146–152.

Hariri AR, Mattay VS, Tessitore A, Kolachana B, *et al.* (2002). Serotonin transporter genetic variation and the response of the human amygdala. *Science* **297**, 400–403.

Harmer C, Heinzen J, O'Sullivan U, Ayres R, *et al.* (2008). Dissociable effects of acute antidepressant drug administration on subjective and emotional processing measures in healthy volunteers. *Psychopharmacology* **199**, 495–502.

Harmer CJ, Bhagwagar Z, Perrett DI, Völlm BA, *et al.* (2003). Acute SSRI administration affects the processing of social cues in healthy volunteers. *Neuropsychopharmacology* **28**, 152.

Harmer CJ, Goodwin GM, Cowen PJ (2009). Why do antidepressants take so long to work? A cognitive neuropsychological model of antidepressant drug action. *British Journal of Psychiatry* **195**, 102–108.

Harmer CJ, Shelley NC, Cowen PJ, Goodwin GM (2004). Increased positive *vs.* negative affective perception and memory in healthy volunteers following selective serotonin and norepinephrine reuptake inhibition. *American Journal of Psychiatry* **161**, 1256–1263.

Hayward G, Goodwin GM, Cowen PJ, Harmer CJ (2005). Low-dose tryptophan depletion in recovered depressed patients induces changes in cognitive processing without depressive symptoms. *Biological Psychiatry* **57**, 517–524.

Heils A, Teufel A, Petri S, Stöber G, *et al.* (1996). Allelic variation of human serotonin transporter gene expression. *Journal of Neurochemistry* **66**, 2621–2624.

Heinz A, Braus DF, Smolka MN, Wrase J, *et al.* (2004). Amygdala-prefrontal coupling depends on a genetic variation of the serotonin transporter. *Nature Neuroscience* **8**, 20–21.

Hu X, Oroszi G, Chun J, Smith TL, *et al.* (2005). An expanded evaluation of the relationship of four alleles to the level of response to alcohol and the alcoholism risk. *Alcoholism: Clinical and Experimental Research* **29**, 8–16.

Hu XZ, Lipsky RH, Zhu G, Akhtar LA, *et al.* (2006). Serotonin transporter promoter gain-of-function genotypes are linked to obsessive–compulsive disorder. *American Journal of Human Genetics* **78**, 815–826.

Hu XZ, Rush AJ, Charney D, Wilson AF, *et al.* (2007). Association between a functional serotonin transporter promoter polymorphism and citalopram treatment in adult outpatients with major depression. *Archives of General Psychiatry* **64**, 783–792.

Keil A, Ihssen N (2004). Identification facilitation for emotionally arousing verbs during the attentional blink. *Emotion* **4**, 23–35.

Kucera H, Francis W (1967). *Computational Analysis of Present-Day American English*. Providence: Brown University Press.

Kwang T, Wells TT, McGeary JE, Swann WB, *et al.* (2010). Association of the serotonin transporter promoter region polymorphism with biased attention for negative word stimuli. *Depression and Anxiety* **27**, 746–751.

Leppanen JM (2006). Emotional information processing in mood disorders: a review of behavioral and neuroimaging findings. *Current Opinion in Psychiatry* **19**, 34–39.

Lesch K-P, Bengel D, Heils A, Sabol S, *et al.* (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* **274**, 1527–1531.

Lesch KP, Gutknecht L (2005). Pharmacogenetics of the serotonin transporter. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **29**, 1062–1073.

Markus CR, De Raedt R (2011). Differential effects of 5-HTTLPR genotypes on inhibition of negative emotional information following acute stress exposure and tryptophan challenge. *Neuropsychopharmacology* **36**, 819–826.

Markus CR, Firk C (2009). Differential effects of tri-allelic 5-HTTLPR polymorphisms in healthy subjects on mood and stress performance after tryptophan challenge. *Neuropsychopharmacology* **34**, 2667–2674.

McGaugh JL (2004). The amygdala modulates the consolidation of memories of emotionally arousing experiences. *Annual Review of Neuroscience* **27**, 1–28.

McMahon FJ, Buervenich S, Charney D, Lipsky R, *et al.* (2006). Variation in the gene encoding the serotonin 2A receptor is associated with outcome of antidepressant treatment. *American Journal of Human Genetics* **78**, 804–814.

Mongeau R, Blier P, Montigny C (1993). *In vivo* electrophysiological evidence for tonic activation by endogenous noradrenaline of α2-adrenoceptors on 5-hydroxytryptamine terminals in the rat hippocampus. *Naunyn-Schmiedeberg's Archives of Pharmacology* **347**, 266–272.

Moore JH (2008). Analysis of gene–gene interactions. *Current Protocols in Human Genetics* (Suppl. 59), ■, 1–10. AQ5

Munafò MR, Brown SM, Hariri AR (2008). Serotonin transporter (5-HTTLPR) genotype and amygdala activation: a meta-analysis. *Biological Psychiatry* **63**, 852–857.

Munafò MR, Johnstone EC, Mackintosh B (2005). Association of serotonin transporter genotype with selective processing of smoking-related stimuli in current smokers and ex-smokers. *Nicotine and Tobacco Research* **7**, 773–778.

Murphy F, Smith B, Cowen P, Robbins T, *et al.* (2002). The effects of tryptophan depletion on cognitive and affective processing in healthy volunteers. *Psychopharmacology* **163**, 42–53.

Murphy SE, Norbury R, O'Sullivan U, Cowen PJ, *et al.* (2009*a*). Effect of a single dose of citalopram on amygdala response to emotional faces. *British Journal of Psychiatry* **194**, 535–540.

Murphy SE, Yiend J, Lester KJ, Cowen PJ, *et al.* (2009*b*). Short-term serotonergic but not noradrenergic antidepressant administration reduces attentional vigilance to threat in healthy volunteers. *International Journal of Neuropsychopharmacology* **12**, 169–179.

Murthy NV, Selvaraj S, Cowen PJ, Bhagwagar Z, *et al.* (2010). Serotonin transporter polymorphisms (SLC6A4 insertion/deletion and rs25531) do not affect the availability of 5-HTT to [11C] DASB binding in the living human brain. *NeuroImage* **52**, 50–54.

NCI-NHGRI Working Group on Replication in Association Studies (2007). Replicating genotype-phenotype associations. *Nature* **447**, 655–660.

Nelson H (1982). *The National Adult Reading Test* (*NART*). Windsor: NEFR-Nelson.

Norbury R, Mackay CE, Cowen PJ, Goodwin GM, *et al.* (2007). Short-term antidepressant treatment and facial processing: functional magnetic resonance imaging study. *British Journal of Psychiatry* **190**, 531–532.

Nutt DJ (2002). The neuropharmacology of serotonin and noradrenaline in depression. *International Clinical Psychopharmacology* **17**, S1–S12.

Osinsky R, Reuter M, Kupper Y, Schmitz A, *et al.* (2008). Variation in the serotonin transporter gene modulates selective attention to threat. *Emotion* **8**, 584–588.

Paddock S, Laje G, Charney D, Rush AJ, *et al.* (2007). Association of GRIK4 with outcome of antidepressant treatment in the STAR*D cohort. *American Journal of Psychiatry* **164**, 1181–1188.

Pezawas L, Meyer-Lindenberg A, Drabant EM, Verchinski B, *et al.* (2005). 5-HTTLPR polymorphism impacts human cingulate–amygdala interactions: a genetic susceptibility mechanism for depression. *Nature Neuroscience* **8**, 828–834.

Praschak-Rieder N, Kennedy J, Wilson AA, Hussey D, *et al.* (2007). Novel 5-HTTLPR allele associates with higher serotonin transporter binding in putamen: a [^{11}C]DASB positron emission tomography study. *Biological Psychiatry* **62**, 327–331.

Rasch B, Spalek K, Buholzer S, Luechinger R, *et al.* (2009). A genetic variation of the noradrenergic system is related to differential amygdala activation during encoding of emotional memories. *Proceedings of the National Academy of Sciences USA* **106**, 19191–19196.

Rawlings N, Norbury R, Cowen P, Harmer C (2010). A single dose of mirtazapine modulates neural responses to emotional faces in healthy people. *Psychopharmacology* **212**, 625–634.

Raymond JE, Shapiro KL, Arnell KM (1992). Temporary suppression of visual processing in an RSVP task: an attentional blink? *Journal of Experimental Psychology: Human Perception and Performance* **18**, 849–860.

Reimold M, Smolka MN, Schumann G, Zimmer A, *et al.* (2007). Midbrain serotonin transporter binding potential measured with [^{11}C]DASB is affected by serotonin transporter genotype. *Journal of Neural Transmission* **114**, 635–639.

Roiser JP, Müller U, Clark L, Sahakian BJ (2007). The effects of acute tryptophan depletion and serotonin transporter polymorphism on emotional processing in memory and attention. *International Journal of Neuropsychopharmacology* **10**, 449–461.

Routledge C, Middlemiss D (1996). The 5HT hypothesis of depression revisited. *Molecular Psychiatry* **1**, 437.

Rubinsztein J, Rogers R, Riedel WJ, Mehta MA, *et al.* (2001). Acute dietary tryptophan depletion impairs maintenance of 'affective set' and delayed visual recognition in healthy volunteers. *Psychopharmacology* **154**, 319–326.

Schosser A, Kasper S (2009). The role of pharmacogenetics in the treatment of depression and anxiety disorders. *International Clinical Psychopharmacology* 24.

Serretti A, Kato M, De Ronchi D, Kinoshita T (2007). Meta-analysis of serotonin transporter gene promoter polymorphism (5-HTTLPR) association with selective serotonin reuptake inhibitor efficacy in depressed patients. *Molecular Psychiatry* **12**, 247–257.

Small KM, Brown KM, Forbes SL, Liggett SB (2001). Polymorphic deletion of three intracellular acidic residues of the alpha 2B-adrenergic receptor decreases g protein-coupled receptor kinase-mediated phosphorylation and desensitization. *Journal of Biological Chemistry* **276**, 4917–4922.

Souery D, Amsterdam J, de Montigny C, Lecrubier Y, *et al.* (1999). Treatment resistant depression: methodological overview and operational criteria. *European Neuropsychopharmacology* **9**, 83–91.

Todd RM, Anderson AK (2009). The neurogenetics of remembering emotions past. *Proceedings of the National Academy of Sciences USA* **106**, 18881–18882.

Todd RM, Palombo DJ, Levine B, Anderson AK (2011). Genetic differences in emotionally enhanced memory. *Neuropsychologia* **49**, 734–744.

Uher R, Huezo-Diaz P, Perroud N, Smith R, *et al.* (2009). Genetic predictors of response to antidepressants in the GENDEP project. *Pharmacogenomics Journal* **9**, 225–233.

van Dyck CH, Malison RT, Staley JK, Jacobsen LK, *et al.* (2004). Central serotonin transporter availability measured with [^{123}I]β-CIT SPECT in relation to serotonin transporter genotype. *American Journal of Psychiatry* **161**, 525–531.

Vuilleumier P (2005). How brains beware: neural mechanisms of emotional attention. *Trends in Cognitive Sciences* **9**, 585–594.

Wakeno MM, Kato MM, Okugawa GM, Fukuda TP, *et al.* (2008). The alpha 2A-adrenergic receptor gene polymorphism modifies antidepressant responses to milnacipran. *Journal of Clinical Psychopharmacology* **28**, 518–524.

Wendland JR, Martin BJ, Kruse MR, Lesch KP, *et al.* (2006). Simultaneous genotyping of four functional loci of human SLC6A4, with a reappraisal of 5-HTTLPR and rs25531. *Molecular Psychiatry* **11**, 224–226.

Willeit M, Praschak-Rieder N, Neumeister A, Pirker W, *et al.* (2000). [^{123}I]-[beta]-CIT SPECT imaging shows reduced brain serotonin transporter availability in drug-free depressed patients with seasonal affective disorder. *Biological Psychiatry* **47**, 482–489.