The Serotonin Transporter Gene Alters Sensitivity to Attention Bias Modification: Evidence for a Plasticity Gene

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Background: Attention bias modification (ABM) procedures have been shown to modify biased attention with important implications for emotional vulnerability and resilience. The use of ABM to reduce potentially toxic biases, for instance, is a newly emerging therapy for anxiety disorders. A separate line of gene-by-environment interaction research proposes that many so-called vulnerability genes or risk alleles are better seen as plasticity genes, as they seem to make individuals more susceptible to environmental influences for better and for worse.

Methods: A standard ABM procedure was used with a sample of 116 healthy adults. Participants were randomly assigned to one of two training groups. One received an ABM procedure designed to induce a bias in attention toward negative material, while the other was trained toward positive pictures. Individuals with low- and high-expressing forms of the serotonin transporter gene (5-HTTLPR) were compared.

Results: Those with a low-expression form (S/S, S/Lg, or Lg/Lg) of the 5-HTTLPR gene developed stronger biases for both negative and positive affective pictures relative to those with the high-expression (La/La) form of the gene.

Conclusions: Here, we report the first evidence that allelic variation in the promotor region of the 5-HTTLPR gene predicts different degrees of sensitivity to ABM. These results suggest a potential cognitive mechanism for the gene-by-environment interactions that have been found in relation to the serotonin transporter gene. Variation on this genotype may therefore determine who will benefit most (and least) from therapeutic interventions, adversity, and supportive environments.

Key Words: Anxiety, attention bias modification, cognitive bias, genetics, GxE interaction, serotonin transporter gene

The search for vulnerability genes or risk alleles has been central to the field of psychiatric genetics. It now seems that while specific genes are unlikely to be linked in a direct way to psychopathology, they do moderate the impact that the environment has on stress sensitivity (1–3). Evidence for such gene-by-environment (GxE) interactions, in spite of ongoing controversy, has been gaining momentum. Central to this debate is the burgeoning number of studies examining a repeat length polymorphism in the promotor region of the human serotonin transporter gene (*5-HTT*, *SLC6A4*), which has become the most widely studied genetic variant in psychiatry, psychology, and neuroscience (4–9). The short (s) allelic form of the serotonin transporter-linked polymorphic region (5-HTTLPR) is associated with reduced activity of the serotonin transporter, resulting in higher levels of intrasynaptic serotonin (low expression) compared with the long (l) form, which leads to reduced levels of intrasynaptic serotonin (high expression) (3,8).

In 1996, it was reported that the s allele was associated with increased self-reports of trait-anxiety or neuroticism, a personality construct known to be linked with increased risk of depression (10). Then, in 2003, an influential longitudinal study found that carriers of the s allele were indeed at increased risk of depression and suicidality but only if they had experienced serious stressful life events or childhood abuse (11). This classic GxE interaction led to a burgeoning of research that remains controversial (4–6,12). While some meta-analyses find that the GxE effects do not hold up across studies (12), others find that as long as a detailed and comprehensive analysis of stressful life events is documented, the 5-HTTLPR short variant does moderate the impact of life stress on psychopathology (6,7). Thus, when extensive details are taken with regard to life events, such as relationship breakups, etc. in one-to-one interviews, GxE effects are strong, while they are often not detected when such specifics are not obtained (4).

Another factor that may contribute to the difficulty of replication in this field is the possibility that the s allele actually increases sensitivity to the environment in a more general way so that adverse environments will lead to bad outcomes, while positive and supportive environments will lead to benefits. In other words, the s allele may not be a vulnerability genotype so much as a plasticity genotype (13–15). Uher (2) has argued that one explanation as to why so-called risk alleles have been conserved throughout evolution might be because the social context shapes the outcome of these essentially neutral genetic factors. In other words, more malleable neural circuits can lead to negative outcomes under adversity but also hold the potential for positive gains when the environment is supportive. This means that the neural circuits relating to the processing of affective significance, which are controlled to some extent by the serotonergic system, may be sensitized in s-allele carriers (16). The 5-HTTLPR short variant may, therefore, act as a plasticity gene that renders individuals more susceptible to environmental influences for better and for worse (13–15). It is worth noting, however, that negative material has a stronger draw on attention than does positive material (17). This means that attentional biases to negative, especially threat-related, material is generally stronger than biases toward positive information when compared with a neutral baseline. Thus, while plasticity may operate to both negative and positive information, attention will generally be more responsive to the negative.

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A separate line of research shows that biases to selectively process threat-related, relative to positive or benign, information is a risk factor for psychopathology. For example, automatic selective biases to direct attention toward negative material better predicts stress reactivity 4 months later, as measured by cortisol response, relative to standardized measures of neuroticism and trait-anxiety (18). It is, therefore, unsurprising that s-allele carriers usually demonstrate increased attentional bias for threat (19–26), which has been confirmed in a recent meta-analysis, and increased amygdala reactivity to threat-related images (27,28). Of particular interest, a recent study shows that s-allele carriers are faster than l homozygotes to pick up fear responses in a fear-conditioning paradigm (29), supporting the notion that people with this genotype are more sensitive to fear-related cues in the environment. Because fear learning is a primary mechanism through which attentional biases for threat develop (30,31), we can speculate that this may be one mechanism through which s-allele carriers acquire a bias toward the more negative aspects of the environment.

New techniques to actively induce or modify attentional biases provide a unique methodology to test the hypothesis that s-allele carriers' heightened sensitivity to threat results in the development of potentially toxic biases that leave them more susceptible to psychopathology. MacLeod *et al.* (32) first demonstrated that selective biases in attention could be modified by a simple computerized technique and that induction of a threat bias leads to increased stress reactivity, whereas the induction of a benign bias leads to a reduction in emotional vulnerability. These findings are important, as they provide evidence for the causal nature of biased attention in stress vulnerability; an experimentally induced bias changes stress reactivity. Their attention bias modification (ABM) technique involved participants being required to identify a nonemotional probe, such as a letter or a symbol, that could appear in one of two locations on the computer screen immediately following the presentation of two words, one of which was negative (e.g., failure, humiliation) and one of which was neutral (e.g., carpet). To train attention toward negative words, the critical probe always appeared in the location previously occupied by a negative word, whereas to induce a benign bias, the probe always appeared in the location previously occupied by a neutral word. Variants of this ABM task have been tested in a range of anxiety disorders and have been shown to reduce threat-related biases and produce marked improvements in clinical symptoms (33–35). Attention bias modification techniques demonstrate that attentional biases are highly plastic and might provide novel treatment strategies for anxiety disorders (35).

The present study presents the first investigation of the hypothesis that carriers of the short variant of the 5-HTTLPR will be more responsive to ABM interventions. We used a novel form of the ABM task that presented only positive and negative pictures, rather than comparing each with a neutral item. The main reason for this was because the wider literature on ABM conflates valence and arousal. Because we wanted to isolate the effects of valence (negative and positive material), we used well-validated pictorial stimuli that were matched for arousal level. This would not have been possible if a neutral control had been included on each trial. Based on previous findings with fear conditioning (29), we expected s-allele carriers to develop stronger biases for threat in an ABM task when compared with those homozygous for the l allele. Moreover, if the s allele really does confer greater sensitivity to the environment for better and for worse, then we would also expect stronger development of a positive bias for pleasant images in people with this genotype. In contrast, if the s allele is better characterized as a vulnerability gene primarily responsive to fear-relevant information, then sensitivity to environmental contingencies should occur only with threat-related stimuli.

Methods and Materials

Participants

Participants were recruited from a pre-existing database at the University of Essex if they carried either the low-expression (i.e., S/S, S/Lg, or Lg/Lg) or the high-expression (La/La) variant of the 5-HTTLPR gene. Sixty-two participants with the low-expression and 54 with the high-expression genotype were recruited. None had a prior or current psychiatric diagnosis and all reported taking no medication that might affect their mental ability. All had normal or corrected-to-normal vision and gave written informed consent to participate in the study. For the low-expression group, 31 participants were randomly assigned to an attention training procedure to induce attentional bias toward negative images (negative ABM), while 31 were assigned to a training condition designed to induce bias toward positive images (positive ABM). For the high-expression genotype group, 26 participants were assigned to the negative ABM, while 26 were assigned to positive ABM. Participants were either paid £6 or awarded course credit for taking part in the experiment.

Genotyping of Serotonin Transporter Polymorphism

For DNA collection, participants provided three to four eyebrow hairs with their root ball intact, which were placed into a labeled 1.5 mL tube and centrifuged. DNA was extracted using the Qiagen (Qiagen GmbH, Hilden, Germany) DNeasy blood and tissue kit according to the manufacturer's instructions, using 180 uL of ATL buffer plus 20 uL of proteinase K for the extraction (both, Qiagen). DNA was eluted in 200 uL AE buffer from the Qiagen columns and stored at −20°C until analyzed. The samples were assayed with a combined polymerase chain reaction (PCR)/restriction digest procedure that enabled the distinguishing of three alleles of the serotonin transporter, a length polymorphism (long and short alleles), and a single nucleotide polymorphism (SNP) within the long allele of the locus. The following two primers were used for the PCR (the forward primer carries a 6-FAM label at the 5′ end):

IDna5HTTP1FF	Fam-CCCAGCAACTCCCTGTACCCCTCCTA
IDna5HTTPA4R	CGCAAGGTGGGCGGGAGGCT

Qiagen Type-It microsatellite PCR mix was used for the PCR amplification, using a final volume of 10 uL. Each PCR contained 2.5 uL DNA (or water for control subjects), 5 uL of 2 × PCR mix, 1 uL Q reagent, 1 uL of primers, and .5 uL of water. The final primer concentration was 200 nmol/L each primer. The PCR mixes were cycled using the following scheme: hot start at 95°C for 5 minutes; 40 cycles of 94°C for 30 seconds, 68°C for 90 seconds, and 72°C for 90 seconds; then a final extension at 60°C for 30 minutes. An aliquot of the PCR products was diluted 1:40 with water, then 1 uL mixed with 9 uL of formamide containing Rox500 GeneScan molecular weight markers (Applied Biosystems, Foster City, California). Samples were analyzed by capillary electrophoresis in an Applied Biosystems 3730 instrument, enabling the distinguishing of the long allele (351 bases) from the short allele (307 bases).

A second aliquot of 2 uL of the PCR products was digested with the HpaII restriction enzyme in a reaction volume of 20 uL, with 1 unit of enzyme, at 37°C for >90 minutes (Invitrogen, Carlsbad, California). Digest products were diluted 1:40 as before, mixed with formamide plus markers, and separated as above. The sizes of bands generated were 259 bases (long allele plus A SNP base), 217 (short allele), and 86 bases (long allele plus G SNP base).