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BRIEF REPORT

The Orienting of Spatial Attention to Backward Masked Fearful Faces Is Associated With Variation in the Serotonin Transporter Gene

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Threat signals facilitate spatial attention, even when awareness of these signals has been restricted through the use of backward masking. However, unrestricted/unmasked threat cues tend to delay the disengagement of attention, whereas restricted/masked threat facilitates orienting, suggesting different underlying mechanisms. Within the general population, the serotonin transporter gene polymorphism (*5HTTLPR*) is associated with one's allocation of attention to unmasked threat signals. However, it is unclear to what extent the *5HTTLPR* gene may be involved in nonconscious biases to masked threat, and whether or not such biases are driven by facilitated orienting or delayed disengagement. Participants were genotyped and performed a dot-probe task with backward masked fearful and neutral faces. Results indicate that short-allele carriers of the *5HTTLPR* gene nonconsciously orient spatial attention to masked fearful faces. On the other hand, homozygous long-allele individuals tended to direct attention away from masked fearful faces. All participants' performance was at chance in a posttask assessment of awareness for the masked faces. The results add to current literature on the *5HTTLPR* and attention biases, and suggest that threat signals facilitate the orienting of attention in short-allele carriers of the *5HTTLPR* gene even under restricted processing conditions.

Keywords: spatial attention, backward masking, faces, nonconscious, attention bias

Within one's environment, salient exogenous visual signals are preferentially processed and facilitate covert spatial attention. This capture of spatial attention can be divided into three subprocesses: (a) orienting or shifting to a new stimulus, (b) engaging or focusing on a stimulus, and (c) disengaging or releasing focus from a stimulus (Posner, 1980). In the general population and particularly in anxious individuals, visual signals of threat exogenously capture spatial attention both in unrestricted processing conditions (MacLeod & Mathews, 1988; Mogg & Bradley, 1999) and when awareness has been restricted through the use of backward masking (Carlson & Reinke, 2008; Fox, 2002; Mogg & Bradley, 2002).[1] The capture of spatial attention by unmasked/unrestricted threat images is primarily associated with a delay in the disengagement of attention from the threat location (Cooper & Langton, 2006; Fox, Russo, Bowles, & Dutton, 2001; Koster, Crombez, Verschuere, & De Houwer, 2004; Koster, Crombez, Verschuere, Van Damme, & Wiersema, 2006; Yiend & Mathews, 2001) and appears to be driven by a frontoparietal cortical attention network (Armony & Dolan, 2002; Pourtois, Schwartz, Seghier, Lazeyras, & Vuilleumier, 2006). On the other hand, masked/restricted threat cues consistently facilitate the orienting of spatial attention (Carlson & Reinke, 2008, 2010) and appear to be mediated by an amygdalo–anterior cingulate network in adults (Carlson, Reinke, & Habib, 2009) and the amygdala in children with anxiety (Monk et al., 2008). Therefore, both restricted and unrestricted threat signals facilitate spatial attention, but appear to do so through separate neural systems and attentional subprocesses.

Research has begun to explore how one's genetic makeup may be associated with preferential biases in spatial attention to negatively or positively valenced emotional stimuli. For example, 5-year-old monozygotic twin pairs tend to share more similar attention biases to fearful and happy facial expressions (either toward or away) compared with dyzygotic twin pairs of the same age, suggesting an early genetic influence on this behavior (Elam,

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[1] Backward masking consists of a brief initial stimulus presentation closely followed by a second "masking" stimulus, which is thought to interrupt and replace the processing of the initial stimulus (Enns & Di Lollo, 2000).

Carlson, DiLalla, & Reinke, 2010). The mechanism underlying this genetic influence could be tied to a functional polymorphism in the promoter region of the serotonin transporter gene (*5HTTLPR*). In neuroimaging studies, short (S)-allele carriers (i.e., SS and SL genotypes) compared with homozygous long (LL) individuals are characterized by greater amygdala reactivity to threatening faces (for review, see Hariri & Holmes, 2006). Behavioral research indicates that genetic variation in the *5HTTLPR* is associated with attentional biases to emotional stimuli in both adolescent (Perez-Edgar et al., 2010) and adult samples (Beevers, Gibb, McGeary, & Miller, 2007; Fox, Ridgewell, & Ashwin, 2009; Kwang, Wells, McGeary, Swann, & Beevers, 2010; Osinsky et al., 2008). Studies using unrestricted/unmasked stimuli have found that *5HTTLPR* S-allele carriers preferentially allocate attention to threat signals (Osinsky et al., 2008; Perez-Edgar et al., 2010) and LL individuals are biased to attend to positive stimuli or away from threatening stimuli (Fox et al., 2009; Kwang et al., 2010; Perez-Edgar et al., 2010). A single study reported an association between *5HTTLPR* S-allele carriers and attention bias to restricted/masked threatening words in a psychiatric sample, but it did not include a control group or assess participant awareness (Beevers et al., 2007). In sum, the literature on biased attention to threat signals implicates a role of genetics—and the *5HTTLPR* gene in particular.

Yet, it is currently unclear to what extent the *5HTTLPR* genotype is associated with *nonconscious* biases toward threatening stimuli within the general population. Furthermore, to our knowledge no study has assessed which particular subcomponent(s) of spatial attention are associated with the *5HTTLPR* genotype. The aim of the current study was to address these two untested aspects of the relationship between *5HTTLPR* genotype and attentional bias toward threat. We hypothesized that backward-masked fearful faces would facilitate the orienting of covert spatial attention among individuals who carry the short allele of the *5HTTLPR*.

Method

Participants

Fifty-one individuals (30 men) between the ages of 19 and 45 years (M = 21.96 years, SD = 3.84) participated in the study. Forty-five individuals reported being right-handed, and six reported being left-handed. Participants were compensated for their time ($20/hr). The Institutional Review Board of Stony Brook University approved this study.

Genotyping

The genotyping procedure used here has been described previously (Olvet, Hatchwell, & Hajcak, 2010). Briefly, the Quick Extract DNA Extraction Solution (Epicenter Technologies, Madison, WI) was used for DNA extraction from buccal cells. Methods were adapted from Wendland, Martin, Kruse, Lesch, and Murphy (2006) to identify *5HTTLPR/rs25531* (S_A, S_G, L_A, and L_G) genotypes. Reaction conditions contained the following steps: (a) initial denaturation at 95 °C for 15 min, (b) 94 °C for 30 s, (c) 68.1 °C for 90 s, and (d) 72 °C for 60 s, and (d) 72 °C for 10 min. Next, 7 μl of polymerase chain reaction product were digested by HpaII (5 U; New England Biolabs, Ipswich, MA) in a 20 μl reaction containing 1 × NEBuffer 1 and 1 × bovine serum albumin at 37 °C for 3 hr.

Our sample included 41 S-allele carriers (SS/SL_A: M age = 21.63 years; 23 men, 37 right-handed), which consisted of 19 SS (M age = 20.89 years) and 22 SL_A (M age = 22.27 years) individuals. Note that the L_G allele was treated as an "S" allele (Wendland et al., 2006). The homozygous L_AL_A group contained 10 individuals (M age = 23.30 years; 7 men, 9 right-handed). Using the Hardy–Weinberg equilibrium calculator (Rodriguez, Gaunt, & Day, 2009), our genotype distribution did not deviate from the expected distribution, $\chi^2(1) = 0.61$, $p > .1$.

Procedure

The task was programmed in E-Prime and was presented on a 60 Hz 16-in. PC computer monitor. Four (two female) grayscale faces depicting fearful and neutral expressions were used for the masked faces, and a fifth (female) open-mouthed happy facial expression from the same facial database (Gur et al., 2002) was used as the mask. Each trial started with a white fixation cue (+) centered on a black background for 1,000 ms. Then two face stimuli were simultaneously presented (33 ms) to the left and right of fixation. Facial stimuli subtended approximately 5 × 7° of visual angle and were separated by 14° of visual angle. After 33 ms, the faces were masked with an open-mouth happy expression (100 ms). Immediately after the mask, a target dot was presented in the location of either the left or the right face and remained until the participant responded. Using a keyboard numeric pad, participants were instructed to identify the location of the dot as quickly as possible by pressing the *1* key with their right index finger for left-sided targets and pressing the *2* key with their right middle finger for right-sided targets. The fixation cue remained in the center of the screen throughout each trial. Participants were instructed to always fixate on this cue.

Directed spatial attention trials consisted of one fearful and one neutral face, half of which were congruent (target dot presented on the same side as the fearful face) and half incongruent (target dot presented on the same side as the neutral face). Faster reaction times (RTs) on congruent compared with incongruent trials indicate a capture of spatial attention. We also included an undirected (neutral–neutral) baseline condition. On these trials, attention should not be preferentially directed to either side of the screen. This baseline condition was used to assess whether the congruent versus incongruent attention effect was driven by rapid orienting to threat (i.e., faster RTs on congruent compared with baseline), delayed disengagement from threat (i.e., faster RTs on baseline compared with incongruent), or a combination of these effects (e.g., see Carlson & Reinke, 2008; Koster et al., 2004). There were 40 congruent, 40 incongruent (counterbalanced for visual field), and 40 neutral–neutral trials randomly presented in a unique order for each participant.

After the dot-probe task, participants completed a task to assess awareness of the experimental stimuli. This task was identical to the dot-probe task in all aspects except that after the backward masking procedure participants were asked to indicate with a keyboard press whether they saw (a) a fearful face on the left, (b) a fearful face on the right, or (c) two neutral faces. Prior to beginning this task, participants were told that each trial would contain two sets of faces presented in rapid

succession and that they were to identify the facial expressions of the first set of faces. Note that the facial identities and visual angles used in this task were identical to those in the dot-probe task, and that the three possible trial types were the same as those used in the dot-probe task. The task included 60 random trials, 20 of each type.

Results

Awareness Check

One of the 51 participants performed significantly better than chance (i.e., 33.33%) on the awareness check task ($M = 45.00\%$, $SE = 6.50$), $t(59) = 1.80$, $p_{\text{one-tailed}} < .05$. This individual (S-carrier) was excluded from all additional analyses. The average performance on the awareness check task for the remaining 50 individuals was at chance (Group $M = 33.94\%$ correct, $SE = 0.47$), $t(49) = 0.93$, $p_{\text{one-tailed}} > .1$. Accuracy did not differ between S-allele carriers ($M = 34.13\%$, $SE = 0.07$) and LL individuals ($M = 33.20\%$ correct, $SE = 0.16$), $t(48) = -0.56$, $p > .1$.

Dot-Probe Task

Analyses were preformed on correct responses occurring between 150 and 750 ms after target presentation (Carlson & Reinke, 2008). As a result, 4.3% of the data were discarded, and 95.7% were used for analysis. A 2 × 2 × 2 mixed model analysis of variance was conducted to assess the effects of visual field (left vs. right), congruency (congruent vs. incongruent), and *5HTTLPR* genotype (S-allele carriers vs. LL individuals) on participants' RTs during directed attention conditions in the dot-probe task. There was a significant Congruency × *5HTTLPR* Genotype interaction, $F(1, 48) = 10.11$, $p = .003$, $\eta_p^2 = .17$. As displayed in Figure 1a, follow-up pairwise comparisons indicate that S-allele carriers had faster RTs on congruent ($M = 390.54$ ms, $SE = 7.86$) compared with incongruent ($M = 398.66$ ms, $SE = 8.07$ ms) trials ($p = .003$, $d = -0.53$), whereas LL individuals had faster RTs on incongruent ($M = 414.81$ ms, $SE = 16.15$) compared with congruent ($M = 425.13$ ms, $SE = 15.73$) trials ($p = .05$, $d = 0.52$).[2] No other effects were significant.

To identify the specific component(s) of spatial attention underlying the observed attention bias to backward masked fearful faces in *5HTTLPR* S-allele carriers, we followed up with *t* tests comparing congruent and incongruent trials with the neutral–neutral baseline. As can be seen in Figure 1b, for S-allele carriers, RTs on congruent trials ($M = 390.54$ ms, $SE = 6.03$) were faster than baseline ($M = 397.97$ ms, $SE = 6.89$), $t(39) = -3.06$, $p = .004$, $d = -0.52$, whereas incongruent trials ($M = 398.66$ ms, $SE = 6.50$, $d = 0.05$) did not differ from baseline, $t(39) = -0.27$. For LL individuals, congruent ($M = 425.13$ ms, $SE = 26.25$), $t(9) = -0.90$, $p > .10$, $d = 0.29$, and incongruent ($M = 414.81$ ms, $SE = 25.64$), $t(9) = 1.22$, $p > .10$, $d = 0.40$, RTs did not differ from baseline ($M = 419.62$ ms, $SE = 26.12$).

Discussion

We found that backward-masked fearful faces captured spatial attention among S-allele carriers of the *5HTTLPR* gene. By including a neutral-only baseline condition, we were able to specifically attribute this effect to a preferential facilitation in orienting. In a small sample of LL individuals, we found that attention was directed away from masked fearful faces. Overall, these results add to prior work suggesting that *5HTTLPR* genotype plays a role in attentional biases toward unmasked threatening stimuli (Fox et al., 2009; Kwang et al., 2010; Osinsky et al., 2008; Perez-Edgar et al., 2010) and to masked threat in psychiatric populations (Beevers et al., 2007). The results are also consistent with prior work reporting that attentional capture to masked threat is driven by facilitated orienting to threat (Carlson & Reinke, 2008, 2010). Thus, our results are consistent with prior work, but extend this work by revealing the first evidence that *5HTTLPR* S-allele carriers orient spatial attention to threat even when those stimuli are masked and participants are unable to detect their expressions.

Although the exact mechanism in which *5HTTLPR* S-allele carriers are biased to rapidly orient spatial attention to threat is unknown, recent neuroimaging results provide intriguing evidence for an altered amygdalo-anterior cingulate attention network. Functional imaging research suggests that *5HTTLPR* S-allele carriers have a hyperactive amygdala response to threat images (Hariri & Holmes, 2006). Anatomically, *5HTTLPR* S-allele carriers have a reduction in anterior cingulate cortex gray matter compared with LL individuals (Pezawas et al., 2005), which appears to coincide with both hyperactive and hypoactive functional coupling between distinct anterior cingulate cortex subregions and the amygdala in response to threat (Heinz et al., 2005; Pezawas et al., 2005). Other neuroimaging research suggests that the amygdala is activated during the facilitation of attention to restricted threat signals (Carlson et al., 2009; Monk et al., 2008) and this attention-related amygdala activation has been found to positively correlate with activation in the anterior cingulate cortex (Carlson et al., 2009). Thus, *5HTTLPR* S-allele carriers appear to have differential processing in a system that has been implicated in mediating a rapid orienting to masked threat. Collectively, this may suggest that a unique amygdalo-cingulate coupling in *5HTTLPR* S-allele carriers is at least partially related to nonconscious biases in spatial attention to masked threat. However, further research directly assessing the neural mechanisms associated with the observed facilitation in spatial attention to masked threat in *5HTTLPR* S-allele carriers is needed.

A limitation of the current study is the relatively small sample for genetic analyses, which may have limited our ability to detect small to medium effects, which can lead to inconstancies in the literature. This issue is particularly relevant for our LL group. Although our finding of directed attention away from threat images in LL individuals is consistent with prior work using unmasked threat (Fox et al., 2009; Kwang et al., 2010; Perez-Edgar et al., 2010), further research with larger samples of LL individuals using masked stimuli is needed. On the other hand, the number of S-allele carriers in this study was relatively substantial and, therefore, the results relating to these individuals can be interpreted more confidently. In addition, we used only masked fearful faces;

[2] Reaction times were similarly faster for congruent (SS: $M = 383.38$ ms, $SE = 9.37$; SL: $M = 397.02$ ms, $SE = 7.69$) compared with incongruent (SS: $M = 390.37$ ms, $SE = 9.37$; SL: $M = 406.16$ ms, $SE = 7.72$) and baseline (SS: $M = 387.58^*$ ms, $SE = 10.66$; SL: $M = 407.36$ ms, $SE = 8.64$) trials for the SS and SL groups ($ps_{\text{one-tailed}} < .05$. * $p_{\text{one-tailed}} = .09$).

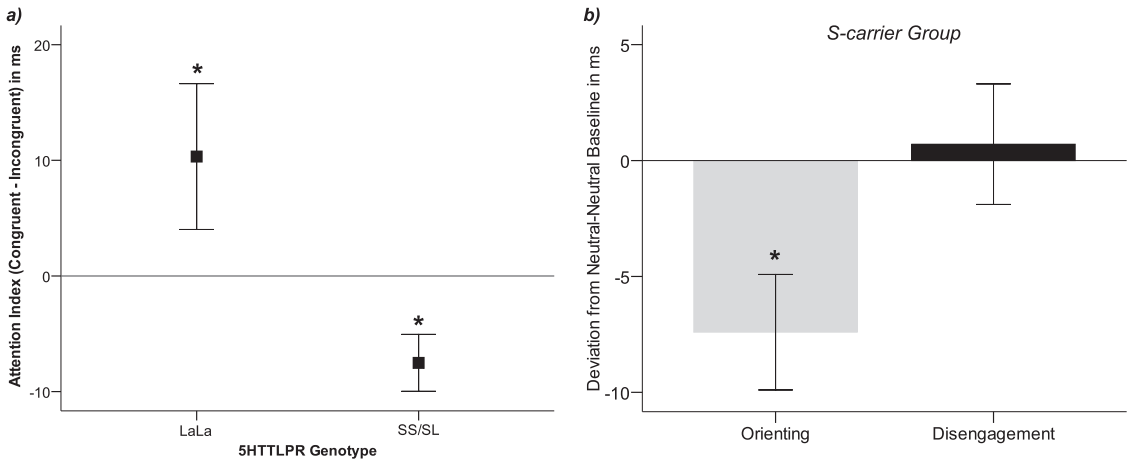

Figure 1. (a) In the dot-probe task, S-allele carriers of the *5HTTLPR* genotype responded faster on fearful face congruent (vs. incongruent) trials, indicating a preferential attention bias to threat, whereas L_AL_A individuals responded faster on incongruent trials, suggesting a bias away from threat. (b) The attention bias to masked fearful faces in the S-carrier group is driven by a rapid orienting toward threat (congruent – baseline) rather than a difficulty in disengaging from threat (incongruent – baseline).

thus, it is unclear whether other facial expressions or unmasked faces would show similar associations between the *5HTTLPR* gene and the orienting of spatial attention. Therefore, although further research is needed on the genetic make-up of affective attention bias, we have provided initial evidence linking the short allele of the *5HTTLPR* gene to a nonconscious orienting response toward backward masked fearful faces.

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