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Effects of *HTR1A* rs6295 polymorphism on emotional attentional blink

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People prone to mood disorders and anxiety typically show increased sensitivity to task-irrelevant stimulation signifying threat. Better knowledge about the brain mechanisms mediating this sensitivity as well as about individual inherited differences in how these mechanisms function is a precondition for developing improved vulnerability screening, resilience building and treatment methods. The chances to have affective disorders are known to depend, among other factors, on the functioning of the brain serotonin systems developed under influence from common genetic variability. However, the extent and directions of the effects of SNPs involved in serotonergic regulation on the propensity for suboptimal threat-sensitivity are poorly understood. This applies also to *HTR1A* rs6295 polymorphism. Assisted by our custom developed emotional attentional blink task, we found that nonclinical subjects carrying the G allele (compared to C allele homozygotes) had higher sensitivity to threat-depicting distractor stimuli, expressed as an increase in the blink magnitude. We also disrupted right-hemisphere dorsolateral prefrontal cortex by rTMS (repetitive transcranial magnetic stimulation) to look for the possible role of DLPFC (dorsolateral prefrontal cortex; known to be involved in cognitive control of responses to affective stimuli) in serotonergic regulation mediated by the *HTR1A* rs6295 polymorphism. No main effects or interactions with rTMS being involved were found.

Key words: threat detection, affect, vulnerability, *HTR1A*, single nucleotide polymorphisms, serotonin, attentional blink

INTRODUCTION

As we are constantly bombarded with emotionally loaded sensory information, coping with emotionally adverse environmental influences is key to establishing a healthy mental life. Psychological vulnerabilities emerge when emotion regulation mechanisms have become disturbed or are suboptimal due to one's life experience and/or unfavourable genetic predispositions, often leading to neuropsychiatric disorders, such as depression, anxiety or even psychosis (Zilverstand et al., 2017). A typical and well-known manifestation of such adversities is hypersensitivity to signals that involve or predict some form of threat which can lead to emotional reactions that are difficult to control (Mathews et al., 1997; Bar-Haim et al., 2005; Koster et al., 2006; Cisler and Koster, 2010; Schulz et al., 2013; Markovic et al., 2014). Early detection of such predispositional vulnerabilities could prove useful for being able to offer timely and efficient assistance for preventing the development of mood disorders.

Behavioral control and regulation of affect depend on gene-environment interactions and encompass both cortical and subcortical structures where genetic variance is expressed (Todd et al., 2013; Markovic et al., 2014; Hibar et al., 2015; Bogdan et al., 2016; Xu et al., 2017). Monoaminergic brain systems, including the serotonin neurotransmission and -modulation systems, are known to be implicated in anxiety- and mood disorders such as depression, and associated with common genetic variability of the genes influencing 5-HT dependent brain processes (Le Francois et al., 2008; Harro et al., 2009; Homberg and Lesch, 2011; Lesch, 2011; Newman-Tancredi and Albert, 2012; Asan et al., 2013; Fabbri et al., 2013; De Deurwaerdere and

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Giovanni, 2017; Lörincz and Adamantidis, 2017; Wilson et al., 2018; Albert et al., 2019). From animal as well as human studies it has become clear that individuals characterized by affective disorders show heightened sensitivity to real or contextually forecast threat. This threat sensitivity has been linked with the possession of the so-called risk variants of the 5-HT related genes (Bigos et al., 2008; Osinsky et al., 2008; Tops et al., 2009; Fisher et al., 2011; Miu et al., 2012; Fisher and Hariri, 2013; Homberg et al., 2016; Kraehenmann et al., 2016; Kroes et al., 2019). One of the genes that has been implicated in the pathophysiology of anxiety and depression is the serotonin 1A receptor encoding gene *HTR1A* (Lemonde et al., 2003; Albert et al., 2019). The *HTR1A* gene encodes the 5-HT1A receptor that plays a major role in a variety of behavioral domains and has been implicated in the development of psychiatric disorders, including depression (Lesch and Gutknecht, 2004; Drago et al., 2008; Langenecker et al., 2019). The 5-HT1A receptors are widely distributed as postsynaptic serotonin receptors in the brain, but also play a strategic role as somatodendritic autoreceptors in the raphe nuclei (Albert and Vahid-Ansari, 2019). This dual role in the regulation of serotonin release which has attracted much attention in drug development has yet to be understood completely (Sniecikowska et al., 2019).

One of the most common SNPs of the *HTR1A* that has been proposed as a candidate for the early detection of vulnerability to mood disorders is the functional C-1019G variant (rs6295) (Le Francois et al., 2008; Albert et al., 2019). More specifically, possessing the G allele or having G/G homozygosity has been considered a risk genotype as these variants have been found to be associated with major depression and resistance to the antidepressant medication. Nevertheless, the results of the studies have been controversial and inconsistent (Wilson et al., 2018). For example, Benedetti and colleagues (2011) found the G homozygotes to be less resilient to stressful factors, consistently with other studies indicating G as the risk allele (Lemonde et al., 2003). On the other hand, Gonzalez-Castro et al. (2013) in their meta-analysis of nine studies showed that the rs6295 polymorphism was not related to vulnerability. In the majority of studies with humans the rs6295 G allele association with threat sensitivity has been shown in clinical or preclinical samples, but the effects are not always clear in studies with the general population (Chipman et al., 2010). However, it is important that data is also collected from neurotypical population subjects to develop early genotyping tests of potential vulnerability. This is obvious from the perspective of the advancement of societal health-care policies by genotyping based screening practices.

Activity in the dorso-lateral prefrontal cortex (DLPFC) has been shown to be involved in the control of attention when responding to threatening stimuli in the context of emotional attentional blink (EAB) assessment (Peers et al., 2013). It is also known that depression is characterized by hypoactivity of the DLPFC (Groenewold et al., 2013) and targeting DLPFC with non-invasive neuromodulation such as transcranial direct current stimulation (tDCS) or transcranial magnetic stimulation (TMS) has been often used for depression treatment (Pascual-Leone et al., 1996; Randver, 2018). Therefore, it should be of no surprise that in the majority of attempts to manipulate emotional self-regulation and resilience by means of non-invasive neuromodulation the DLPFC has been targeted, both for nonclinical research purposes and clinical treatment purposes (Grall-Bronnec and Sauvaget, 2014; Sagliano et al., 2016; Berger et al., 2017; Naish et al., 2018). For instance, a study by Sanchez and colleagues (2016) demonstrated that targeting the right DLPFC with TMS stimulation caused impairments in attentional disengagement from both positive and negative faces. However, while the number of studies is already substantial, there is no consensus about the reliability and extent of the TMS effect on DLPFC. Whether the effect has been obtained depends on several factors, such as frequency of stimulation (1-Hz inhibitory protocol *vs.* above 5 Hz excitatory protocol, or even single-pulse online stimulation protocols), laterality of TMS application (right hemisphere *vs.* left hemisphere *vs.* bilateral TMS application), individual anxiety levels of the participants, the type of vulnerability involved, etc. For example, concerning the efficacy of rTMS treatment of addiction related vulnerability, seven out of eighteen treatments had no effect (Grall-Bronnec and Sauvaget, 2014). In a different analysis, Naish et al. (2018) observed that in ten cases non-invasive modulation had an effect while in another ten cases the effect was absent. In one work (Malaguti et al., 2011) TMS/DLPFC was investigated specifically in relation to the *HTR1A* rs6295 polymorphism. They showed that patients with C/C genotype showed a greater improvement after DLPFC/TMS facilitative stimulation than those with G/G and C/G genotypes, which suggests higher resilience in C/C and/or supports the known increased vulnerability of G allele carriers (Albert et al, 2019). It is possible that individual differences indicated by genetic polymorphisms modulate the effects of TMS on DLPFC, explaining some of the controversial results in the literature.

As individual levels of the EAB effect could be a marker of emotional vulnerability in relation to *HTR1A* genotype and because there is a continuing quest for non-invasive neuromodulatory treatment methods, TMS effects on the level of expression of EAB

is an important avenue of study. While the EAB test has proven to be a quite robust method for measuring sensitivity to emotive stimuli, we have not come across research where the effects of DLPFC-targeted TMS on the "emotion induced blindness" (Most et al., 2005; Wang et al., 2012) have been studied in relation to *HTR1A* variability. Some researchers have found an interaction between the 5-HT1A receptor genotype and TMS stimulation in the context of sensitivity to the treatment of major depression by medication: C/C patients showed a higher difference between active and sham stimulation, indicating that these patients benefited more from TMS than those belonging to C/G and G/G group (Zanardi et al., 2007). For our purposes it is essential to note that rTMS can modulate serotonergic transmission by desensitizing 5-HT1A autoreceptors (Gur et al., 2000); the effect was found for both the presynaptic 5-HT1A autoreceptors situated somatodendritically in the raphe nuclei and the 5-HT1B autoreceptors situated on nerve terminals.

However, the study by Gur et al. (2000) was conducted on rodents. Although Malaguti et al. (2011) used rTMS targeted to human DLPFC, the target was located in the left-hemisphere DLPFC. At the same time, we know that 5-HT1A-related cortical endophenotypes are especially associated with the cognitive control of affective reactions and not so much with cognitive processing *per se*. We also know that specifically the right DLPFC exerts control over impulsive, automatic reactions to distractive affective cues (Miller and Cohen, 2001; Cromheeke and Mueller, 2014; Banich et al., 2019), leading to affective/attentional biases toward negative information (Plewnia et al., 2015; Salehinejad et al., 2017). All this brings us to the need to study the automaticity of (maladaptive) affective reactivity as a function of both 5-HT1A genetic variability and manipulated level of right DLPFC functional state. As healthy people are likely to have developed skills for coping with aversive stimuli, their threat sensitivity has to be assessed with a method shown to produce robust effects also in general population. Conveniently, EAB qualifies as such method. As far as we know, healthy rs6295 subjects outside clinical or preclinical samples have not been investigated in the context of EAB and target perception before.

The present study has three main aims: (1) by using the EAB paradigm, to assess individual sensitivity to affective stimuli in relation to genetic polymorphisms in the 5-HT1A gene; (2) by using the EAB paradigm, to explore whether repetitive transcranial magnetic stimulation (rTMS) targeted at right DLPFC can modulate sensitivity to aversive stimuli; (3) to explore whether (if at all) rTMS/DLPFC effects interact with *HTR1A* rs6295 variants.

METHOD

Sample and ethics

For participation in the EAB experiment and genotyping, 67 participants were recruited via mailing lists of universities in Tallinn and Tartu (age range 19-50). Two prospective participants were rejected due to performing poorly in the task (below 20% discrimination rate) and two more did not finish the task due to discomfort from TMS. The final sample included 63 participants (23 male, 40 female), ages ranged from 19 to 40 (mean=24.92, SD=3.86). All participants were healthy and had normal or corrected to normal vision. Participants gave informed consent before participating in the experiment. Research was approved by the Research Ethics Committee and was conducted according to the principles stated in the Declaration of Helsinki.

Procedure

Participants were assigned to the TMS or SHAM group based on age and gender, to keep both groups as comparable as possible. After signing the informed consent sheets, participants were seated in a dimly lit room in front of the computer monitor and fitted with an EEG cap. The EEG cap was kept on for the duration of the experiment in order to accurately deliver TMS pulses to the assigned locations. EEG electrodes F4 and Cz were used for the experimental and control condition, respectively. The F4 has previously been used to locate the right DLPFC for TMS studies (Karton et al., 2014). Applying stimulation to the vertex (electrode Cz) is a common control condition for TMS studies, as this area has relatively little influence over ongoing brain processes and was therefore used as the SHAM condition in terms of producing audible clicks of the stimulator, sensations caused by TMS cutaneously, and participant knowledge that they are stimulated by TMS in all experimental conditions.

Prior to TMS stimulation, participants were introduced to the procedure and completed 20 practice trials to learn the task. They were instructed to maintain gaze fixation at the centre of the screen and assured that this was the most effective way to complete the task successfully. After ascertaining that the participants had understood the task, 5 min (300 s) of 1-Hz rTMS stimulation followed. TMS stimulation was presented at 47% of maximum intensity which is the average of individual thresholds used in previous comparable experiments (Karton et al., 2014; Rutiku et al., 2016). Five minutes of stimulation was estimated to be

sufficient to induce the desired neural effects while minimizing possible confounding activation (Fitzgerald et al, 2006).

Each trial started with the presentation of a fixation cross at the centre of the screen for 500 ms, followed by a picture stimulus (distractor, 10 deg of visual angle) which was either neutral or threatening/emotional, presented for 300 ms. These stimuli were selected from the widely used International Affective Picture System, based on their arousal ratings (IAPS; Lang et al., 1997). The 48 negative stimuli had an average arousal rating of 6.57 (range 5.62-7.29) and the 48 neutral images had an average arousal rating of 2.97 (range 1.74-3.46). Each unique distractor stimulus was presented a total of four times per experiment. Immediately following the distractor stimulus, a sequence of 16 different spatially overlapping characters - letters and a number (1.8 degrees of visual angle) - appeared unpredictably either to the right or left side of the central fixation locus (350 px from the centre) in a rapid serial visual presentation (RSVP) stream, while participants maintained fixation. They were instructed to identify a number (1-9) within the stream of letters. The number was presented either at the 2nd or 5th location in the sequence. The RSVP stream was presented with 10-Hz frequency (100 ms each character). Stimuli were presented on a white background (46 cd/m^2). One in seven trials (drawn at random) were catch trials where no number appeared. Participants were instructed to attempt to guess the number even if they were unsure, but if they were certain that they had not seen a number, to report 0. Following the question to identify the number, participants were asked to rate on a four-point PAS type scale (Overgaard et al., 2006) how clearly they saw the number (0 - did not see the number at all, 1 - not so sure if I saw the number I reported, 2 - quite sure I saw the number I reported, 3 - very certain I saw the number I reported).

Gene data analysis

Following the experiment, a saliva sample was collected from each subject, using SalivaGene Collection Module II (STRATEC Biomedical AG) kits. Genotyping of the acquired DNA was carried out at the Department of Neuropsychopharmacology at the University of Tartu.

Genomic DNA was extracted from saliva samples using MN NucleoSpin® Blood Kit (740951.250; MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany) The real-time polymerase chain reaction (RT-PCR) for genotyping the *HTR1A* rs6295 polymorphism was performed using a TaqMan Pre- Designed SNP Genotyping Assay (Applied Biosystems; Foster City, CA, USA).

C_11904666_10 containing primers and fluorescent probes. Genotyping reactions were performed in a total volume of 10 µl with ~25 ng of template DNA. RT-PCR reaction components and final concentrations were as follows: 1:5 5 × HOT FIREPol® Probe qPCR Mix Plus (ROX) (Solis BioDyne) and 1:20 80 × TaqMan Primers Probe. Context sequence [VIC/FAM] was as follows:

ATGGAAGAAGACCGAGTGTGTCTTC[C/G]
TTTTTAAAAAGCTACCTCCGTTCTC

Reactions were performed on the Applied Biosystems ViiA™ 7 Real-Time PCR System. The amplification procedure consisted of an initial denaturation step at 95°C for 12 min and 40 cycles of 95°C for 15 s and 60°C for 1 min. Positive and negative controls were added to each reaction plate. No inconsistencies occurred. Genotyping was performed blind to all phenotypic data.

Behavioral data analysis

The behavioral data was analyzed using repeated measures ANOVA; to reduce the effect of outliers without losing data nonparametric tests were preferred for the comparison of group means. All data analyses were performed using R software (https://www.R-project.org/; version 3.3.0).

RESULTS

Behavioral results

Across our sample (N=63) the average percentage of correct answers in the objective discrimination task was 74.7% (SD=11.1%) and the average subjective clarity rating (PAS score) was 1.60 (SD=0.47). All targets (numbers 1-9) were discriminated well above chance (between 59% and 83% on average). Objective response accuracy and subjective rating were significantly correlated in all conditions (r between 0.44 and 0.70, all $p<0.001$).

To explore other possible effects and interactions between the various experimental conditions, a repeated measures ANOVA was conducted with the factors emotional content of stimulus (neutral/negative), target location (2nd/5th in the sequence), sequence position (left/right) and experimental block (1st and 2nd half). The TMS condition was included as a between-subjects factor, although no effects between the two experimen-

tal groups emerged. There was a main effect of stimulus content (F(61)=57.06, p<0.001, ges=0.016), target location (F(61)=31.08, p<0.001, ges=0.108) and block (F(61)=55.63, p<0.001, ges=0.032) on response accuracy (percent of correct answers).

Participants made more mistakes after negative images had been presented (72.39% correct) than after neutral images (77.03% correct), which confirms that negative threatening images had a stronger effect for inducing emotional attentional blindness compared to neutral distractor images. More mistakes were also made when the target was presented at the 2nd location of the sequence (68.40% correct) compared to when it appeared 5th (80.94% correct) – as expected, most of our sample experienced attentional blink around 200 ms after the T1 stimulus, which is a commonly reported SOA to obtain the blink for attentional blink experiments. Performance improved in the second block (78.15%) compared to the first block (71.18%), which implies that there was a learning effect to this task.

There was a significant interaction between stimulus content and target location (F(61)=63.74, p<0.001, ges=0.016) - the blink was larger for emotional stimuli compared to neutral stimuli when the target was presented 2nd, but there was no difference between stimulus content when the target was presented 5th, which is to be expected as most individuals experienced a decrease in performance at the 2nd not 5th location of the letter sequence. Thus, our subjects were susceptible to the version of emotional attentional blink (EAB) developed for our study (see Fig. 1).

There was an interaction between block and stimulus content (F(61)=11.91, p=0.001, ges=0.003) as well as between block and target location (F(61)=6.97, p=0.010, ges=0.003), which showed that the differences between conditions decreased slightly in the second block, as overall performance improved (see Fig. 2)

Lastly, an interaction between RSVP sequence lateral position and target location in the RSVP stream was found (F(61)=20.88, p<0.001, ges=0.013) which showed that the blink effect was more pronounced if the RSVP stream was presented to the left visual field.

Analogous results were obtained for subjective answers (PAS score) There was a main effect of stimulus content (F(61)=39.64, p<0.001, ges=0.007), target location (F(61)=23.93, p<0.001, ges=0.054) and block (F(61)=27.34, p<0.001, ges=0.019).

Although there was no difference in response accuracy between trials where the sequence appeared on the left versus where it appeared on the right side of the screen (F(61)=4.04, p=0.841), there was a small but significant difference in subjective answers (F(61)=5.18, p=0.026, ges=0.006), as participants were more confident about targets which were presented on the left side of the screen (M=1.65) than on the right side (M=1.56).

There were significant interactions between stimulus content and target location (F(61)=73.10, p<0.001, ges=0.007), stimulus content and block (F(61)=13.26, p<0.001, ges=0.002) and target location and sequence position (F(61)=13.45, p<0.001, ges=0.004). Subjective visibility level shows similar regularities to the objective discriminability level: EAB is apparent at the earlier RSVP position and not at the later RSVP position of the target (Fig. 3). A preceding affective stimulus causes a decrease in subjective vividness of the affectively neutral target object selectively at the typical attentional blink temporal position, supporting the notion that an affective stimulus transiently „steals“ some share of the attentional resources from perceptual processing of the additional stimuli.

In general, the task was effective for inducing EAB - the task-irrelevant negative stimulus presented at the beginning of the trial reduced the accuracy of target detection compared to trials where a neutral image was presented.

Effects of the genetic polymorphism

To explore the potential link between the genetic polymorphism and vulnerability to threatening images, we calculated the size of the emotional blink

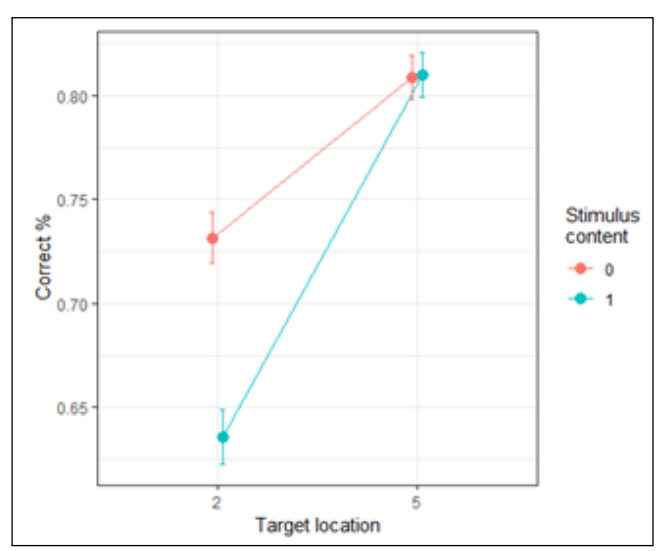

Fig. 1. Proportion of correct discrimination responses of the number characters (targets) as a function of target temporal position in the RSVP stream (2nd and 5th position) and type of the distractor stimuli presented before the RSVP (neutral *vs.* affectively loaded). Affectively loaded distractor stimuli caused a larger decrease in performance in the 2nd target position compared to neutral distractor stimuli. 95% confidence intervals are also depicted.

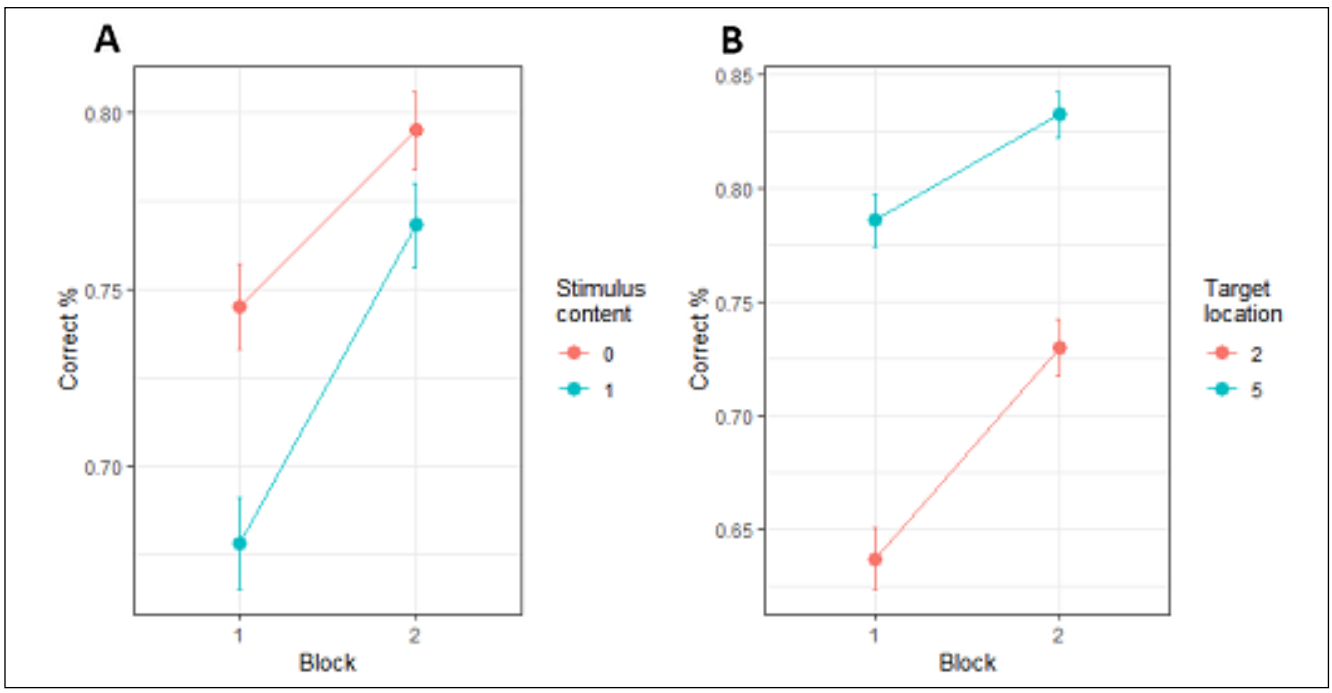

Fig. 2. A (left), proportion of correct discrimination responses of the number characters (targets) as a function of experimental block and type of the distractor stimulus presented before the RSVP (neutral *vs.* affectively loaded); B (right), proportion of correct discrimination responses of the number characters (targets) as a function of experimental block and position of the target in the RSVP stream (2nd and 5th position) 95% confidence intervals are also depicted.

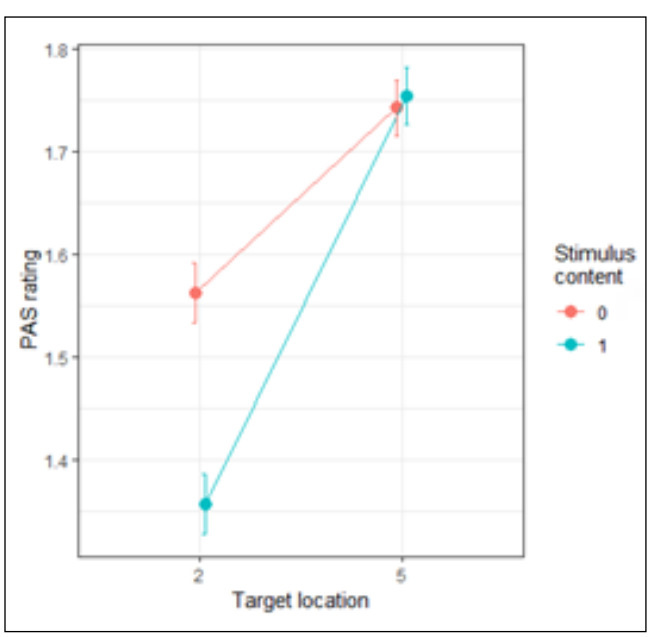

Fig. 3. Average PAS ratings of the subjective vividness of the number character targets as a function of target temporal position in the RSVP stream (2nd and 5th position) and type of the distractor stimuli presented before the RSVP (neutral *vs.* affectively loaded). A preceding affective stimulus causes a decrease in the subjective vividness of the affectively neutral target object selectively at the "blinked" position. 95% confidence intervals are also depicted.

for each participant, i.e. the difference in response accuracy (objective answer) between the emotional (threatening) and neutral distractor image condition in terms of the proportion of correct answers at the critical numeral target location condition. For most participants, the difference in accuracy between the emotional and neutral distractor condition was greater at the 2nd sequence position, but for nine participants the difference was greater at the 5th sequence position. To reduce the effect of outliers, the nonparametric Wilcoxon rank sum test was applied. For this analysis we divided the participants into two groups: C homozygotes, i.e. C/C genotypes (17 participants: 5 M, 12 F; average age 25.3, SD=3.53) and G allele carriers, i.e. G/G and C/G genotypes (46 participants: 18 M, 28 F; average age 24.8, SD=3.99) We found evidence that the *HTR1A* polymorphism had a significant effect on the magnitude of the emotional attentional blink, as the C homozygotes (n=17) were seemingly less affected by the emotional distractor than G allele carriers (n=46); W=233, p=0.014 (group mean difference in C homozygotes=6.92%, group mean difference in G allele carriers=11.27%; see Fig. 4). As G allele carriers are considered belonging to the higher vulnerability group (Albert et al., 2019), this result supports sensitivity of the EAB to risk genotype in the nonclinical and preclinical

population. There was no effect of the *HTR1A* polymorphism on the respective EAB magnitude for subjective answers (W=377, p=0.836)

The *HTR1A* polymorphism also had a significant effect on the overall discrimination rate (F(61)=5.24, p=0.025, ges=0.076), showing that the accuracy of target perception was significantly lower for G allele carriers than C homozygotes (average response accuracy in C homozygotes=79.84%, average accuracy in G allele carriers=72.84%; see Fig. 5). There was also an interaction with distractor stimulus content, as the difference was larger with threatening images compared to neutral images (F(61)=6.69, p=0.012, ges=0.005).

TMS

TMS did not have an effect on overall performance (F(61)=3.77, p=0.541) nor on the EAB (W=453, p=0.562), adding to the controversial results referred to in the Introduction. Although some of the previous studies had established that stimulating the DLPFC affects depression level or the attentional capture of affective stimuli (Malaguti et al., 2011; Sanchez et al., 2016), we failed to replicate any TMS effects. The lack of TMS effects may depend on differences in the TMS protocols, subject samples, tasks etc. To anticipate the following discussion on the gene effects, we found no interaction of TMS with 5HTR1A genetic variants.

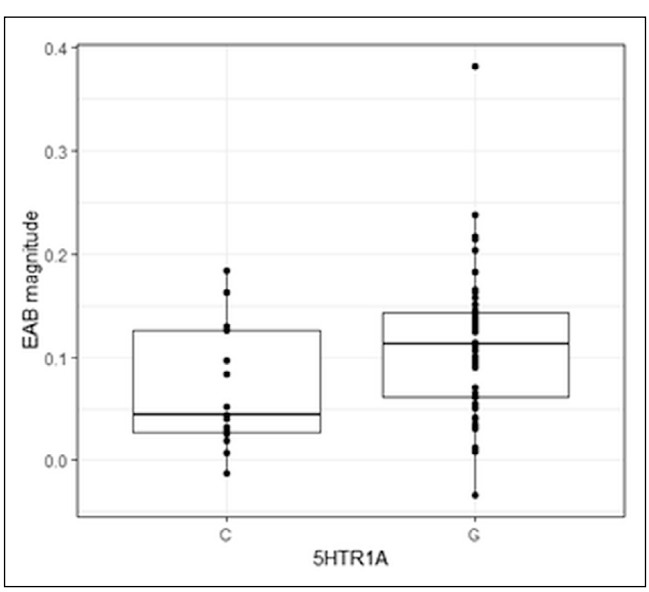

Fig. 4. Difference in the proportion of correct responses between the emotional distractor condition and neutral distractor condition in the emotional attentional blink task (EAB magnitude) for the two genotype groups of the *HTR1A* polymorphism. G allele carriers (GG and CG group) have higher sensitivity to emotional distraction compared to C homozygotes (CC group).

DISCUSSION

In our study, we developed a version of an emotional attentional blink (EAB) task and validated it as an experimental test sensitive to threat-signifying distractor stimuli. The EAB design we used is valid for measuring sensitivity to affectively loaded environmental stimulation in terms of "stealing" attentional resources from the processing of other objects, resulting in decreased target perception selectively at post-distractor delays typical for the standard attentional blink tasks (Dux and Marois, 2009). For most subjects, having a threatening image presented as a task-irrelevant distractor at the beginning of a task-relevant stream of letters had an impairing effect on the discrimination rate (participants performed on average 5% worse in the emotional condition). We also found that the EAB effect was somewhat stronger in the first block of trials compared to the later block. This means that if a threat-sensitivity test similar to the one used here were to be applied for vulnerability testing, the procedure need not be too long, as the effect (if present) is likely to emerge early on.

The successful experimental validation of our EAB task allowed us to pursue the main aim of the present study: to examine the sensitivity of this task to common genetic variability found in a brain serotonergic system related gene (*HTR1A*) for which the association with mood and anxiety related neuropsychiatric disorders is still poorly understood. When considering the

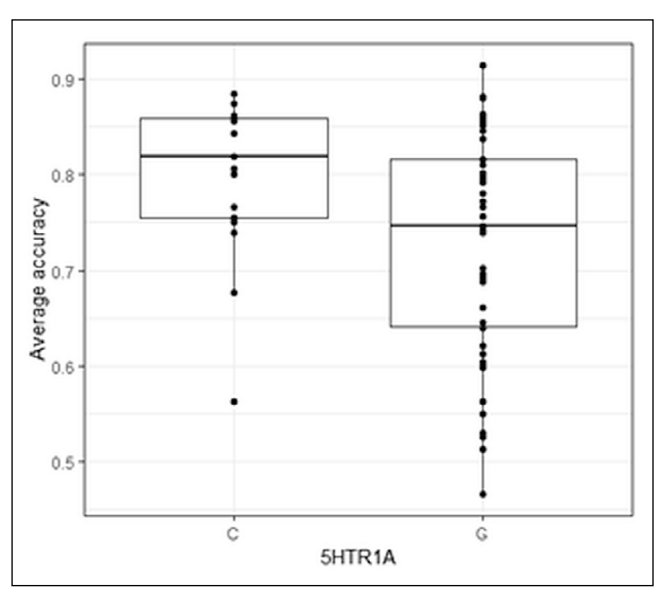

Fig. 5. Difference in the overall proportion of correct responses in the emotional attentional blink task for the two gene groups of the *HTR1A* polymorphism. G allele carriers (GG and CG group) have lower level of target perception accuracy compared to C homozygotes (CC group).

role of the functional C-1019G variant (rs6295) in predicting vulnerability, G allele carriers have been more often than not regarded as more vulnerable (Le Francois et al., 2008; Albert et al., 2019). Yet, some results on the association of *HTR1A* rs6295 with affective vulnerability have been inconsistent and partly controversial (Gonzalez-Castro et al., 2013; Albert et al., 2019). Moreover, in the majority of studies with humans the G allele association with threat sensitivity has been shown in clinical or preclinical samples, but the effects are not always clear in studies with the general population (Chipman et al., 2010). Importantly, in the present experiments we showed that subjects outside clinical or pre-clinical samples with variants of *HTR1A* rs6295 reputedly indicative of vulnerability in terms of proneness to neuropsychiatric mood disorders and anxiety (Benedetti et al., 2011; Lemonde et al., 2003) – i.e., G allele carriers – had stronger EAB and overall lower correct target perception compared to C/C homozygotes. In other words, these subjects (with G/C or G/G genotype) were more disturbed by emotional distractors. Whereas in C homozygotes the capacity for good discrimination of targets presented in a fast stream of spatially overlapping stimuli was better and they were less susceptible to distraction from threat-signalling preceding stimuli.

Our results, which indicate that differences in EAB performance emerge as fast as merely a few hundred milliseconds after exposure to a threat-indicating stimulus, are not too surprising if we look at the data on how fast serotonergic neurons can signal reward and punishment. For example, in a fine study by Cohen et al. (2015) it was demonstrated that serotonergic neurons in mice reacted to reward or punishment within 100-500 ms and the spike burst firing rate differentiated responses to aversive compared to rewarding stimulation. We trust that in humans the time scale of fast responding must not be much different. Nevertheless, precisely how the brain serotonin system is involved in the regulation of attention capture by threat depicting stimuli is not well understood.

There are too many brain systems with serotonin involvement and "serotonergic" gene expression, and their arrangement and interrelations are too complex to present a precise description of the influence on visual perception and attention.The areas include raphe nucleus, frontal cortex, basal forebrain, hypothalamus, midbrain and brainstem structures, and even primary visual cortex. Moreover, as the serotonergic units in the brain have also been shown to exhibit complex interactions with dopaminergic, GABAergic and glutamatergic systems (De Deurwaerdere and Di Giovanni, 2017; Lörincz and Adamantidis, 2017), a precise mechanistic description of the effects on bottom-up invoked perception remains to be elucidated. It is likely that the fronto-parietal attention network is involved, as *HTR1A*, in its interaction with the 5-HTTLPR polymorphism, was found to be associated with the connectivity pattern of this network (Long et al., 2017). In addition, the medial prefrontal cortex and amygdala have been implicated, as 5-HT1A receptors were shown to be involved in amygdala reactivity to threat (Fisher et al., 2011). Among the important tasks for future research, the roles of bottom-up signalling (from V1) and top-down signalling (from frontal cortex downstream), with intermediary in the amygdala, have to be more precisely specified (Kraehenmann et al., 2016). It is already known, for example, that the rs6295 G allele in combination with stress increases amygdala reactivity (Albert et al., 2019), which would be consistent with our present findings.

Interestingly, we did not find an effect of disruptive rTMS targeted at right DLPFC on EAB, which further adds to a literature of conflicting results, although occasionally this type of subject-level effect of noninvasive stimulation has been found (De Raedt et al., 2010; Leyman et al., 2009; London and Slagter, 2015). Furthermore, the TMS condition had no significant behavioral effects or interactions with the *HTR1A* polymorphism. It is possible that one brief session of rTMS was not sufficient to induce any significant effects that would span for the duration of the experiment (approximately 30 min). It should be noted, however, that in all cases the experiment was started within 2 min after finishing the stimulation protocol and no effects were found even when only investigating results from the first or second block separately.

The fact that an effect of the *HTR1A* polymorphism on EAB was found with 63 participants must not cause complacency and increasing sample size in future replication studies to increase power is definitely advisable. Also, studying SNP effects on threat sensitivity and their interaction with DLPFC/TMS with only one serotonergic gene is likely to yield less interesting results compared to polygenic studies purporting to observe interactions and mediational relations between different genes.

CONCLUSION

Despite some persisting inconsistencies and contradictory empirical results as for the involvement of serotonin 1A receptor encoding gene *HTR1A* in predisposing people to anxiety and depression, we added evidence to support this conjecture. Specifically, when examining the functional C-1019G variant (rs6295) effect, we showed that G allele carriers (the genetic "risk variants")

belonging to a nonclinical sample are more sensitive to environmental threat as elicited by IAPS pictures. To demonstrate this, we used a version of an emotional attentional blink type visual task. Subjects who belonged to the "risk group" were more distracted by a threat representing picture. At the same time, we did not find any main effect or interaction with rDLPFC-targeted rTMS involved, casting doubt on the robustness and reliability of TMS effects when attempting to use it for the treatment or conditioning of resilience.

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