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Genetic variation in serotonin transporter function affects human fear expression indexed by fear-potentiated startle

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ABSTRACT

The serotonin transporter (SERT) plays a crucial role in anxiety. Accordingly, variance in SERT functioning appears to constitute an important pathway to individual differences in anxiety. The current study tested the hypothesis that genetic variation in SERT function is associated with variability in the basic reflex physiology of defense. Healthy subjects ($N = 82$) were presented with clearly instructed cues of shock threat and safety to induce robust anxiety reactions. Subjects carrying at least one short allele for the 5-HTTLPR polymorphism showed stronger fear-potentiated startle compared to long allele homozygotes. However, short allele carriers showed no deficit in the downregulation of fear after the offset of threat. These results suggest that natural variation in SERT function affects the magnitude of defensive reactions while not affecting the capacity for fear regulation.

1. Introduction

The serotonin transporter plays a crucial role in anxiety. Accordingly, pharmacological adjustment of serotonin transporter (SERT) functioning by selective serotonin reuptake inhibitors (SSRIs) is among the most established clinical pharmacological strategies to control human anxiety disorders (Baldwin et al., 2005; Bandelow et al., 2002). It is therefore to be expected that genetic variation in SERT function underlies innate differences in individuals' fear reactivity. In the current study we investigated how genetic variance in the SERT gene affects the up and down regulation of basic fear responses.

To this end we studied the impact of a well-known polymorphism in the promoter region of the SERT gene (also called 5-HTTLPR), which influences anxiety-related personality (Lesch et al., 1996). This common 5-HTTLPR polymorphism consists of an insertion/deletion of 43 bp in the 5′ regulatory region of the gene, resulting in either a long or short allele. The short allele is associated with reduced SERT transcriptional activity in vitro (Heils et al., 1996). Meta-analyses have more recently confirmed that carriers of the 5-HTTLPR short allele (S-carriers) report more anxiety related personality traits (Schinka et al., 2004; Sen et al., 2004). However effect sizes are small (Munafo et al., 2009) and the relation between this polymorphism and anxiety- and stress related psychopathology remains a matter of debate (Karg et al., 2011; Lonsdorf et al., 2009a; Wankerl et al., 2010).

A potentially fruitful approach to further investigate sequelae of genetic variability in serotonin transporter function is by establishing intermediate phenotypes associated with the 5-HTTLPR polymorphism (Canli, 2008; Domschke and Dannlowski, 2010). For example, imaging studies showed exacerbated amygdala activation towards fear cues in S-carriers (Hariri et al., 2002; Munafo et al., 2008). Another approach has been to compare 5-HTTLPR genotypes on peripheral psychophysiological measures such as skin conductance and startle during fear conditioning. In a classical conditioning study, S-carriers were more likely to show strong conditioned SCR responses than LL homozygotes who were found more often in a group of subjects that showed weak conditioning (Garpenstrand et al., 2001). More recently, evidence was presented for stronger SCRs in S-carriers during vicarious conditioning (Crisan et al., 2009). Finally, Lonsdorf et al. (2009b) reported that across the acquisition phase of a fear conditioning session, S-carriers showed stronger potentiation of the startle response. Potentiation of the startle reflex is a reliable index of the activation of the defensive system (Bradley et al., 2005) and is widely used as an objective and rather specific measure of fearful responding (Grillon and Baas, 2003; Hamm and Weike, 2005). Taken together these results suggest that increased neural threat processing in S-carriers as witnessed by stronger amygdala activations, may be reflected in autonomic measures of fear and defensive reflexes. Moreover,

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S-carriers may show altered amygdala regulation by the prefrontal cortex (PFC) (Heinz et al., 2005; Pacheco et al., 2009; Pezawas et al., 2005). Since the interaction between these regions mediates fear regulation (Hartley and Phelps, 2010; Quirk and Beer, 2006), this implies that increased anxiety in S-carriers could stem from a genetic deficit in the capacity to suppress fear.

Here we investigated further how the 5-HTTLPR polymorphism affects fear reactions and the downregulation of fear responses. An instructed fear paradigm was employed, in which fear reactions were elicited in healthy volunteers by presenting cues that are identified before the experiment as signaling threat of electric shock (Bocker et al., 2004; Grillon et al., 1991, 1993). Moreover, startle was also measured after the termination of threat cues, during the transition from threat to a period of relative safety. In this way we could index how well subjects of each genotype were able to reduce fear when direct danger subsided. Previous work indicated that the capacity to return to a resting state after threat offset depends on prefrontal-limbic interactions (Klumpers et al., 2010a). Based on the evidence that S-carriers display increased fear reactivity which perhaps relates to altered prefrontal control, we hypothesized that S-carriers would (a) demonstrate greater reactivity to threat cues by showing stronger startle potentiation and (b) would show a reduced capacity for fear regulation as reflected in a slower decline of startle potentiation after the offset of threat cues.

2. Methods and materials

This study was approved by the medical ethical committee at the Utrecht University Medical Centre.

2.1. Subjects

Subjects were recruited through advertisements posted around the faculty and on the faculty website. A total of 95 subjects (aged 18–30) passed inclusion criteria and gave written informed consent. All subjects reported to be free of hearing problems, neurological conditions, cardiovascular disease and psychiatric diagnoses relevant to the current study, including mood and anxiety disorders. Moreover, included subjects reported no regular illicit drug use or use of psychoactive prescription drugs. Of these subjects, 94 completed the instructed fear paradigm. We excluded 11 subjects with missing data, corrupted data and/or minimal startle reactivity based on the criterion that each subject should have at least one artifact free, non-zero response for each condition in each task block (see task description below). In the remaining 83 subjects, we could accurately determine 5-HTTLPR genotype for 82 subjects (60 female; see Table 1). Of subjects these subjects, 76 were Caucasians of European descent. As an analysis excluding other ethnicities did not change the results, we decided to report on the full sample here.

2.2. Genotyping

DNA was collected with buccal swabs and isolated using a standardized kit (QiAmp DNA Mini Kit; Qiagen, Germany). 5-HTTLPR genotyping was performed using polymerase chain reaction (PCR) followed by gel electrophoresis as described by Lonsdorf et al. (2009a; see erratum). This procedure visualized for each subject either 2 short 486 bp DNA fragments (S/S), one short and one long (529 bp) fragment (S/L) or 2 copies of the long fragment (L/L). Out of the 83 subjects, we determined genotype successfully in duplicate for 82 subjects. Genotype percentages were: SS (17%), SL (57%), and LL (26%) and in accordance with the Hardy–Weinberg equilibrium ($p > .5$). Of note, three additional candidate polymorphisms (HTR1A C-1019G, COMT val158met, and DAT1 3′ UTR VNTR) were assessed more exploratorily and did not affect fear-potentiated startle, the reduction in startle after threat offset and the anxiety ratings. Gene–gene interactions were not investigated given that for each interaction, smallest cells contained less than 5 subjects.

2.3. Stimuli and apparatus

Physiological recording and amplification was carried out using the BioSemi Active Two system with matching FLAT active Ag/AgCl electrodes (BioSemi, Amsterdam, The Netherlands). Startle probes were 50-ms white noise bursts with instantaneous rise time presented at 106 dBa through foam in-ear earplugs (Earlink, Aero Company auditory systems, IN, USA). Eye blink startle to these probes was measured by electromyographic (EMG) recordings from the orbicularis oculi. For EMG measurement, electrodes were placed under the right eye; one centralized under the pupil and the other 15 mm lateral towards the outer cantus of the eye. Shocks were administered through a constant current simulator (Digitimer DS7A, Digitimer Ltd., Letchworth Garden City, United Kingdom) with tin cup electrodes located over the upper, inner wrist of the left arm. Two photos of male faces with neutral emotional expression from the NIMSTIM database (Tottenham et al., 2009; models 21 and 23) were used as cues to signal threat and safety in the instructed fear task. One of the photos was presented with blue background and the other with orange background to increase salience and distinctiveness of the cues.

2.4. Procedure

Subjects first completed a medical screening questionnaire and a Dutch translation of the trait portion of the Spielberger State/Trait Anxiety Inventory (STAI; Defares et al., 1980). Electrodes for startle recording and shock administration were applied and the subjects underwent a standardized shock workup procedure consisting of 5 sample shocks to set the shock intensity individually for each subject at a level considered "quite annoying" (see Klumpers et al., 2010a, 2010b). This goal of this procedure was not revealed to subjects to assure an unbiased response. The final intensity of the electrical stimulation varied between subjects from 0.5 to 5.8 mA. After the workup, subjects received instructions about the task (see below). When the instructions were clear to subjects the earplugs were inserted. Twelve startle probes were presented for startle habituation. This initial series of probes was also used as a baseline startle measurement. Immediately after the last habituation probe the instructed fear task commenced.

2.5. Instructed fear task

The task was explained by showing the cue that signaled shock threat for that subject, with the instruction that "at any time during presentation of this cue shocks could be administered". Next subjects were presented with the second cue, and were instructed that they "would never receive shocks" when this cue was presented (Fig. 1). Whether the orange or blue cue signaled shock threat was evenly distributed across subjects. During the task, cues were presented with a variable duration to make the offset of the conditions unpredictable (4–8 s, $M = 5.6$ s). The word "*RUST*" ('rest' in Dutch) was presented on the screen during the intervals between cues. Subjects were instructed to relax during these periods. Rest periods lasted between 6 and 20.5 s ($M = 11.1$ s). Startle probes could be presented at three latencies: (a) during the cues, 3 s after cue onset ("cue probes"), (b) in the rest period following the cue, 1.5 s after cue offset ("offset – early probes") or (c) 5 s after cue offset ("offset – late probes"). Subjects were instructed to ignore the probes as much as possible. The startle probe timing is illustrated in Fig. 1.

The full experiment consisted of 5 experimental blocks with brief breaks after blocks 2 and 4. During these breaks and at the end of the experiment, subjects retrospectively rated their state anxiety during each cue and immediately following the offset of each cue on a computerized scale from 0 (*not anxious*/*nervous*) to 10 (*very anxious*/*nervous*). After the breaks, instructions regarding the threat and safe cue were repeated and 4 startle probes were administered for startle habituation before continuation of the experiment.

Each experimental block contained 10 presentations of each cue. For each condition (threat/safe), 4 cue probes and 4 offset probes (2× offset – early, 2× offset – late) were presented per block. The mean interval with a previous startle probe was kept at 20 s for each of the 6 probe types (2 conditions × 3 probe latencies), with a minimum interval of 16 s after a previous probe or shock reinforcement. A semi-random event order was created, with no more than three consecutive repetitions of the same cue. To exclude order effects, half of the subjects received this event order while for the other half of the subjects threat and safe conditions were presented in the reversed order. This was distributed evenly across genotypes (χ^2 p-value > .6). A total of 9 shocks were administered at varying, semi-random time points to reduce predictability to reinforce the instruction that shocks could be administered at any time during the threat condition.

2.6. Data processing and statistical analysis

Startle data were pre-processed and checked for artifacts according to previously published guidelines (Blumenthal et al., 2005) and procedures (Bocker et al., 2004; Klumpers et al., 2010a, 2010b). To determine baseline startle, startle magnitudes from the habituation trials were log-transformed to correct deviations from normality. For the analysis of the instructed fear task, startle magnitudes for all experimental conditions were transformed to z scores per subject to simultaneously control for impact of baseline startle on fear-potentiated startle. As recommended (Grillon and Baas, 2002), raw data are also reported. Finally, data were averaged according to condition (threat, safe) and probe latency (cue, offset – early and offset – late). Similarly, the state anxiety ratings were averaged according to condition (threat, safe) and latency which only had 2 levels for the rating data (cue, offset).

All the subsequent statistical analyses were carried out in SPSS 17 (SPSS, Chicago, Illinois). Gender was not explicitly matched between genotypes and added as covariate for all genotype comparisons. Consistent with previous research (Brocke et al., 2006; Lesch et al., 1996; Lonsdorf et al., 2009b), we grouped genotypes into S-allele carriers vs. L/L homozygotes to ascertain a minimum sample size of 20 subjects for each group (see Table 1). Genotype groups were compared on trait anxiety, final shock intensity, and baseline startle amplitude in univariate ANOVAs.