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Variation of 5-HTTLPR and Deficits in Emotion Regulation: A Pathway to Risk?

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Genetic variation in the serotonergic system within the brain has been a significant focus of psychiatric research over the last several decades. In particular, the serotonin transporter (5-HTT) gene (*SLC6A4*) has been tied to early emotion processing biases. However, a clear understanding of how genetic variation of SLC6A4 may influence clinically salient emotional phenomena is still elusive. In this investigation, we focused on examining variation in the 5-HTT-linked polymorphic region (5-HTTLPR; including the single nucleotide polymorphism rs25531 which alters genotype interpretation) and real-time emotion responses evoked in the laboratory using a paradigm designed to spontaneously induce emotion regulation. Across 2 studies we show that for healthy individuals with 2 copies of the functional short (S′) allele there is weakened down-regulation of negative emotion. In addition, we found greater electrodermal responses as well as both negative and positive emotion in association with the S′ allele in 1 of the 2 samples. These findings provide evidence that the S′ allele may promote system-wide heightened emotional reactivity in healthy subjects. Both phenomena, observed here in a healthy population, are strongly linked to the development of psychiatric disease. As such, these findings have implications for S′ carriers' vulnerability to affective disorders, as well as suggest potential targets for future clinical investigation.

Keywords: 5-HTTLPR, negative emotion, emotion regulation, psychological risk, serotonergic system

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Unquestionably, genetic variation in the serotonergic system within the brain has been a significant focus of psychiatric research over the last several decades. In particular, the serotonin transporter (5-HTT) gene (SLC6A4) has received the lion's share of research attention in humans. Indeed, recent reviews have been able to integrate and synthesize basic research findings across species that suggest links between variation in SLC6A4 and differences in emotion processing (cf., Hariri & Holmes, 2006; Holmes, 2008). Additional support for the emotional influences of 5-HTT function comes from pharmacological studies exploring the neuroemotional impacts of 5-HTT-inhibiting drugs in humans (Del-Ben et al., 2005; Ma, 2015; Ma et al., 2015; Murphy, Norbury, O'Sullivan, Cowen, & Harmer, 2009). However, despite the considerable research attention focused here, the understanding of how genetic variation of SLC6A4 influences *clinically relevant* differences in emotion that could lead directly to

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emotion-related disorders is still quite limited. Specifically, there is a marked paucity of evidence linking genetic variation of SLC6A4 to clinically salient and behaviorally specific indicators of risk. For example, although there is growing evidence suggesting that behaviorally specific patterns of emotion responses can predict both the onset and persistence of depression (Coifman & Bonanno, 2010; Rottenberg, Kasch, Gross, & Gotlib, 2005), there have not yet been attempts to link these or other related patterns to 5-HTTLPR variation. Indeed, although variation in SLC6A4 has been broadly linked to increased risk for psychiatric diseases, most notably affective disorders (Caspi, Hariri, Holmes, Uher, & Moffitt, 2010; Karg, Burmeister, Shedden, & Sen, 2011), how this actually may manifest in terms of clinically specific vulnerabilities is still largely unknown.

Broadly, the extant research focused on variation in SLC6A4 has linked the functional short (S′) allele of the 5-HTTLPR to heightened negative emotional reactivity.[1] This is largely evident across species and methodology. For example, in humans, most imaging studies link the S′ allele to heightened limbic activity in response to even subliminal exposure to negative emotional stimuli (Hariri et al., 2002; Munafò et al., 2009; Thomason et al., 2010). Moreover, several meta-analyses have converged linking the S′ allele largely (although not always consistently) to heightened neuroticism, which reflects an individual's perception that they are more reactive and attentive to negative content around them (Schinka, Busch, & Robichaux-Keene, 2004; Sen, Burmeister, & Ghosh, 2004). Finally, there are increasing behavioral studies linking the S′ allele to early biased attention to negative emotional cues that often corresponds with disruptions in goal-relevant behavior (Antypa, Cerit, Kruijt, Verhoeven, & Van der Does, 2011; Beevers, Pacheco, Clasen, McGeary, & Schnyer, 2010; Pérez-Edgar et al., 2010).

In other species, the findings are largely consistent. For instance, rhesus macaques carrying the S allele of the 5-HTT gene (rh5-HTTLPR orthologous to the human 5-HTTLPR) display increased neuroendocrine and behavioral responses to stress compared to macaques homozygous for the long (L) allele, an effect that interacts with rearing environment (Barr et al., 2004; Champoux et al., 2002). A large body of research also demonstrates increased anxiety-like behavior, depression-like behavior, and increased stress responses in 5-HTT KO mice, despite considerable debate that 5-HTT KO mice may not always approximate humans homozygous for the S′ allele (cf., Hariri & Holmes, 2006). However, one important finding of the large body of research on 5-HTT KO mice, in particular, is the relevance of genetic variation on brain development. For example, growing evidence suggests that what may be most influenced by 5-HTT gene variation is the nature of the connectivity among key areas highly relevant in emotion and regulatory processing (e.g., amygdala, prefrontal cortex, anterior cingulate, and insula; Dannlowski et al., 2010; Hariri et al., 2002; Ma et al., 2014). Most notable of these is the circuitry linking the amygdala with regions of the prefrontal cortex (PFC, e.g., anterior cingulate PFC; cf., Holmes, 2008; Pezawas et al., 2005). For instance, several human imaging studies have implicated the S′ allele of 5-HTTLPR with reduced or inefficient PFC-amygdala connectivity (Beevers et al., 2010; Gillihan et al., 2010; Heinz et al., 2005; Pezawas et al., 2005), suggesting that individuals with at least one S′ allele may have difficulty down-regulating already heightened emotional responses. Recently, several investigations have demonstrated a link between the S′ allele and increased difficulty disengaging from emotional stimuli via eye-tracking (Beevers et al., 2011; Beevers, Wells, Ellis, & McGeary, 2009). However, to date, there have been no investigations examining the influence of 5-HTTLPR on spontaneous emotion regulatory responses.

Considerable data and theory implicate deficits in emotion regulatory processing as a highly significant risk factor in most psychological disorders, although most clearly in affective disorders (Nolen-Hoeksema & Watkins, 2011). Emotion regulation can be thought of as a broad construct, encompassing the generation or up-regulation of emotion in response to internal or external contexts as well as the down-regulation

[1] Published research has focused predominantly on responses to negative emotional or threat-inducing cues, rather than positive emotion. There are some studies suggesting similar reactivity to positive emotional cues in individuals with copies of the short allele of 5-HTTLPR, although these findings are mixed (e.g., Pérez-Edgar et al., 2010).

of emotion via sometimes automatic or implicit as well as deliberate strategies (Cole, Michel, & Teti, 1994; Gross & John, 2003). Emotion regulation, although often measured via trait-level report, can be rigorously operationalized and assessed by indexing in vivo responses to emotionally salient/evocative contexts in the lab or daily life. Indeed, increasing evidence points to the importance of real-time assessment, since most emotional processing occurs quite rapidly and is outside of awareness (Mauss, Bunge, & Gross, 2007; Williams, Bargh, Nocera, & Gray, 2009), so that sole reliance on reporting (particularly trait-level reports) has been shown to have only limited utility when predicting clinical phenomena (Aldao, Nolen-Hoeksema, & Schweizer, 2010). As such, researchers have increasingly begun to employ emotion induction paradigms that allow for the examination of emotional responses across varying contexts or conditions (Coifman & Bonanno, 2010; Rottenberg et al., 2002) in order to index not just reactivity or the generation of emotion in response to stimuli, but also naturalistic or spontaneous regulation of responses as contextual features change.

In the current investigation, we focused on examining the link between genetic variation in the regulatory region of SLC6A4 (5-HTTLPR) and an associated single nucleotide polymorphism (SNP) rs25531, and emotion regulatory responses in the laboratory. Specifically, in two separate studies, we examined variation in 5-HTTLPR as it influenced patterns of emotion regulation evoked naturalistically in the laboratory in healthy volunteers, in order to begin to better understand the pathway between serotonin transporter function and psychiatric disease. We built upon the growing body of evidence demonstrating the clinical relevance of evaluating spontaneous emotion regulation within and across contextual boundaries, known as emotion context sensitivity (Coifman & Bonanno, 2009; Rottenberg & Gotlib, 2004). In particular, this methodology has proven to be highly predictive of the onset and persistence of emotion-related diseases. As such, our goal was to test to see whether patterns demonstrated to be indicative of vulnerability to psychiatric disease could be predicted by genetic variation. Thereby, identifying a potentially clinically relevant pathway in which variation in SLC6A4 may influence psychiatric risk and laying the foundation for future clinical investigation.

The construct of emotion context sensitivity is grounded in contemporary models of emotion that posit that emotional responses evolved to facilitate adaptation by responding to specific environmental demands. For example, negative emotions, such as sadness, anger, or disgust evolved to facilitate specific challenges (e.g., sadness in response to loss [Bonanno, Goorin, & Coifman, 2008], anger in response to goal-blockage [Lerner & Keltner, 2000], disgust in response to contamination risk [Tybur, Lieberman, Kurzban, & DeScioli, 2013]). As such, adaptive emotional responding and regulation is characterized by flexible engagement and disengagement in response to contextual demands and/or internal needs, or more simply as appropriate responses to a given "incentive context" (Goldsmith & Davidson, 2004).

The emotion context sensitivity framework (ECS) allows for assessment of emotion generated within a context as well as regulatory flexibility across contexts, allowing for considerably greater predictability of the onset (Coifman & Bonanno, 2010) and persistence (Diminich & Bonanno, 2014; Rottenberg et al., 2002) of emotion-related psychiatric disorders as well as behavioral responses under stress (Coifman & Bonanno, 2009; Harvey, Coifman, Ross, Kleinert, & Giardina, 2014). Given the evidence suggesting that variation in SLC6A4 may influence emotion generated within contexts as well as regulatory flexibility across contexts (requiring down-regulation of past responses and generation or up-regulation of new responses), the ECS framework provides an ideal opportunity to test how this variation may influence patterns of emotion processing and regulation shown to contribute to both the onset and persistence of emotion-related disease.

The 5-HTTLPR literature is heterogeneous in terms of analytical approaches, with studies using biallelic or triallelic genotyping, and analyzing data by grouping L/S′ individuals with either L or S′ homozygotes for comparison against the opposing homozygous population, or by evaluating each of the three (or six) genotypes independently. Since the well-known study by Caspi and colleagues (2003), many studies have supported the hypothesis that individuals homozygous for the S′ allele can be considered the most vulnerable or plastic to

environmental influence (Belsky & Pluess, 2009), particularly when objective measures rather than self-reports are employed (Karg et al., 2011; Serretti, Kato, De Ronchi, & Kinoshita, 2007). Based on this information, and in parallel to other studies examining related downstream emotional and/or psychophysiological correlates of 5-HTTLPR genotype (Beevers et al., 2009; Gotlib, Joormann, Minor, & Hallmayer, 2008; McCaffery, Bleil, Pogue-Geile, Ferrell, & Manuck, 2003), we analyzed our data by comparing S′ homozygotes to L carriers.

In Study 1, we examined emotion regulatory responses during four highly emotionally evocative films. In Study 2, we examined emotion regulatory responses to simulated peer-rejection and peer-acceptance using a computer-based paradigm designed and well-validated to induce ostracism: Cyberball (Williams, Cheung, & Choi, 2000). In each study, emotion responses were assessed across multiple dimensions (participant report, coded facial behavior, and Study 2 included autonomic activity) during *and* across multiple contexts in order to index the generation of negative and positive emotion within a given context as well as emotion regulatory shifts across contexts. By employing these two different methodologies to investigate naturalistic emotion regulation during different emotional provocations, we could generate a more cohesive interpretation of 5-HTTLPR genotype influence. Effects observed only in Study 1 or Study 2 could be attributed to differential responding to nonsocial and social emotional contexts, respectively, whereas consistent patterns across studies could be interpreted as meaningful, broader influences of 5-HTTLPR genotype on global emotion processing.

Here, we predicted that individuals carrying two copies of the S′ allele would demonstrate enhanced negative emotion reactivity (greater negative emotion generation) within negative contexts as well as poor down-regulation of negative emotion across contexts (e.g., poor ability to reduce negative emotion responses when the context shifted to positive). Further, we anticipated stronger effects in Study 2 due to the social component, since the S′ allele has repeatedly been associated with greater reactivity in social contexts (Boll & Gamer, 2014; Crişan et al., 2009; Gyurak et al., 2013; Hariri et al., 2005). We did not make a priori hypotheses for positive emotion, given the limited and mostly mixed prior evidence. Nonetheless, we examined similar dimensions of positive emotion.

Method and Materials

Participants

Study 1. Participants were 111 undergraduate students (64% female; 79% Caucasian, 12% African American, 3% Asian, 6% Other; 94% non-Hispanic) recruited from the university community. Mean age was $M = 20.87$, $SD = 6.58$.

Study 2. Participants were 104 undergraduate students (61% female; 88% Caucasian, 8% African American, 4% Asian; 96% non-Hispanic) recruited from the university community. Mean age was $M = 20.48$, $SD = 4.76$.

All participants provided informed consent and were compensated with course credit and both studies were approved by the university Institutional Review Board for Human Subjects Research.

5-HTTLPR Genotyping

Approximately 2 mL saliva was collected from participants and stored at −20 °C until extraction (Beevers et al., 2011). Saliva was extracted using the prepIT-L2P (DNA Genotek inc., Ottawa, Canada), and purification was accomplished using a Genomic DNA Clean & Concentrator kit (Zymo Research, Irvine, CA). Once purified, extracted DNA was quantified with SYBR Green I dye (Lonza, Walkersville, MD), then all samples were diluted to a DNA concentration of 5 ng/μl. Each polymerase chain reaction (PCR) reaction per 1 μl of sample DNA consisted of 5 mM $MgCl_2$, 2 mM dNTPs, 0.5 μmol/L forward and reverse primer, 0.5 μl Taq polymerase, and a final glycerol concentration of 10% vol/vol. The region of the SLC6A4 gene containing the 5-HTTLPR was amplified using a touchdown PCR (Don, Cox, Wainwright, Baker, & Mattick, 1991) on an Eppendorf PCR Mastercycler pro (model no. 6321, Hamburg, Germany). Cycling conditions were adopted and modified from Anchordoquy, McGeary, Liu, Krauter, & Smolen (2003). Only the annealing conditions were altered, such that

annealing was set to 65 °C for 10 cycles, followed by 55 °C for 35 cycles. Primers were designed after the sequence reported by Heils et al. (1996) and designed by Gelernter, Cubells, Kidd, Pakstis, & Kidd (1999) (forward: 5′-ATG CCA GCA CCT AAC CCC TAA TGT – 3′; reverse: 5′-GGA CCG CAA GGT GGG CGG GA- 3′), yielding base pair lengths of 376 (short allele) and 419 (long allele). Amplimers were separated using 1% agarose gel electrophoresis and visualized with ethidium bromide under ultraviolet excitation.

rs25531 SNP Determination

In order to analyze the 5-HTTLPR-specific SNP rs25531, a restriction fragment length polymorphism analysis was run on each PCR sample. The SNP found only among the L carriers was an A or a G, termed the L_A or L_G variant, respectively, which are functionally distinct (Anchordoquy et al., 2003; Hu et al., 2005). In order to discern this SNP, 10 μl of PCR product from above was incubated for one hour at 37 °C in a solution of 1X CutSmart Buffer with 4 U/reaction MspI enzyme (New England BioLabs, Ipswich, MA). In the absence of the L_G SNP, two restriction sites are cut by MspI enzyme, producing products of 283 + 63 + 30 bp (S allele) or 326 + 63 + 30 bp (L_A allele). The L_G allele results in a third MspI site, yielding products of 174 + 152 + 63 + 30 bp. Once analyzed, L_G was classified with the S allele due to similar transcriptional efficiency (Hu et al., 2005). Digested samples were evaluated alongside undigested PCR products as described above. Ten percent of sample genotypes were separately reconfirmed with 100% concordance.

For Study 1, the frequency of the 5-HTTLPR alleles (LL = 43 [39%]; LS = 50 [45%]; SS = 18 [16%]) did not differ from the Hardy-Weinberg equilibrium, $\chi^2 = 0.29$, $p = .87$. For the rs25531 SNP individuals carrying the L_G allele were classified as "S′" resulting in the following allele frequency: LL = 32 (28.8%); LS′ = 51 (45.9%); S′S′ = 28 (25.2).

For Study 2, the frequency of the 5-HTTLPR alleles (LL = 29 [28%]; LS = 53 [51%]; SS = 22 [21%]) also did not differ from the Hardy-Weinberg equilibrium, $\chi^2 = 0.05$, $p = .97$. For the rs25531 SNP individuals carrying the L_G allele were classified as "S′" resulting in the following allele frequency: LL = 23 (22.1%); LS′ = 48 (46.2%); S′S′ = 33 (31.7%).

Questionnaire Measures

Depression symptoms. All participants reported current depression symptoms using the Center for Epidemiological Studies Depression Scale (CES-D) in which scores above 16 reflect clinically significant depression symptoms (Radloff, 1977). We included this measure given considerable prior research demonstrating depression's influence on negative emotional processing and regulation (Gotlib & Joormann, 2010).

Study 1. Mean rated depression CES-D was $M = 11.69$, $SD = 7.52$.

Study 2. Mean rated depression on the CES-D was $M = 11.41$, $SD = 6.95$.

Rejection sensitivity (Study 2 only). Participants in Study 2 also completed the Rejection Sensitivity Questionnaire (Downey & Feldman, 1996) in order to control for dispositional influences on emotion regulation during the Cyberball task. Mean score was $M = 8.36$, $SD = 3.50$, which is consistent with other normative samples (Berenson et al., 2009).

Emotion Response Assessment

As is customary when assessing real-time emotion regulation, assessments were multidimensional in order to capture greater variability responses, as well as to allow for and index positive emotional responses during negative contexts and negative emotion during positive contexts (Coifman & Bonanno, 2010; Rottenberg et al., 2002). *To index emotional experience*, participants made multiple ratings of emotional responses immediately following each context (Study 1: each film clip; Study 2: each of 3 Cyberball games). Participants rated negative emotion words (fear, sadness, disgust, guilt, distress, anger) and positive emotion words (happiness, enjoyment, amusement, affection, relief) on a 1 to 7 Likert scale. Ratings were aggregated by valence to derive scores reflecting negative and positive emotional experiences by film. Internal consistency of word ratings during the four emotionally evocative videos of Study 1 and the three games of Cyberball (Study 2) was high (negative Study 1 $\alpha = .93$, positive Study 1 $\alpha = .91$; negative Study 2 $\alpha = .85$, positive Study 2 $\alpha = .85$). *To index emo-*

tional facial behavior, participant expressions were coded by five research assistants naïve to study details. Following procedures previously established (cf., Bonanno, Papa, Lalande, Westphal, & Coifman, 2004) coders did not receive additional training but instead relied on their natural attunement to emotional expression. Coders viewed participant videos without sound and made global ratings of negative emotional expressions (e.g., please rate how much this person is expressing negative emotion, scale 1 "*very little*" to 7 "*extreme*"), and positive emotional expressions (e.g., please rate how much this person is expressing positive emotion, scale 1 "*very little*" to 7 "*extreme*"), in response to each video clip of each participants viewing of each film or each Cyberball game. Growing evidence has demonstrated that "naïve" coders can be as reliable as highly trained coders (e.g., Dondi et al., 2007). Indeed, our coders were sufficiently reliable (average intraclass correlation coefficient = .80, range .74–.90). Moreover, ratings were averaged across all five coders by participant to increase reliability.

Emotional experience ratings and coded emotional expressions were standardized using *z*-scores and combined by valence of response (negative or positive emotion) yielding two emotion response scores (negative emotion; positive emotion) for each context (Study 1: each film clip; Study 2: each of 3 Cyberball games).[2] In order to capture the ability to regulate emotional responses as film/context changed from negative to positive, responses from negative contexts (i.e., negative films; rejection during Cyberball) were subtracted from responses to the positive contexts (i.e., positive films; acceptance during Cyberball) by valence. As such, each participant also had one change score reflecting negative emotion regulation and one change score reflecting positive emotion regulation.

Finally, in Study 2, we also included *assessment of autonomic responses*, specifically electrodermal responding, an established indicator of sympathetic activation and emotional responsivity (Coifman, Bonanno, Ray, & Gross, 2007). We indexed electrodermal activity (EDA) in real time on the ventral forearm using an Affectiva Q-Sensor (Affdex, Boston, MA; Sano, Picard, & Stickgold, 2014) with 1-cm diameter Ag–AgCl dry electrodes. The sensor logged EDA (in μS), and skin surface temperature at 32 Hz. Data were cleaned and artifacts removed using customized software and visual inspection, yielding scores reflecting the mean level of EDA during each Cyberball game.

Procedures

Study 1. 111 individuals were recruited to complete a study on emotion and attention. After providing informed consent, participants reported current depressive symptoms using the CESD (Radloff, 1977), provided saliva samples (Oragene-DISCOVER OGR-500 kits), and then were seated in front of a 17-in. monitor to begin a naturalistic emotion regulation task that involved watching a series of six previously validated, highly emotionally evocative film clips (Gross & Levenson, 1995; Shaheen, Coifman, Flynn, Matt, & Halachoff, 2014); presented in standardized order using ePrime, 2.0 (Psychology Software Tools, Sharpsburg, PA). Participants were seated comfortably in front of the monitor by themselves and were instructed to engage with films as best they could and following each clip, reported emotional responses. Emotion-related facial behavior was recorded using high-resolution video and coded later by individuals blind to the study details.

Film clips were each approximately 5 min in duration and were ordered to maximize the intensity of elicited responses, as well as to place the greatest demands on participants to spontaneously regulate their emotion responses from clip to clip. The first clip was neutral (*Big Cat Diary*; BBC Earth, 2010) and was included to ensure that participants acclimated to the study demands. The next four clips were presented in a fixed sequence for all participants, alternating negative and positive emotion eliciting films. The first clip elicited intense negative emotions by depicting conflict between inmates and guards (*Road to Guantanamo Bay*; Revolution Films, 2006); and was followed by a positive clip depicting rescue and reunion among close

[2] Combining two dimensions of emotion (here reported experience and coded behavior) yields far more robust representation of emotional responses. However, there are many reasons why dimensions of emotion are sometimes only loosely coupled or concordant. As such, prior to combining coded and experienced emotion we performed within-subject repeated measures ANOVA to test if these emotion indices significantly differed from each other. In both studies, they did not.

friends (*Alive*; Paramount Pictures, 1993), this was followed by another negative clip depicting the loss of a loved one (*The Champ*; Metro-Goldwyn Mayer, 1979) and then another intensely positive clip (*Between Two Ferns*; www.comedyordie.com, 2010). Participants then watched a final, sixth film that was humorous to ensure that mood was restored prior to their departure. Because of the repeated design, affect and facial behavior scores were also aggregated by valence type (i.e., negative vs. positive films).

Study 2. 104 individuals were recruited to complete a study on emotion, attention, and virtual-ball. After providing informed consent, participants reported current depressive symptoms using the CES-D (Radloff, 1977) as well as dispositional sensitivity to rejection (Rejection Sensitivity Questionnaire; Downey & Feldman, 1996) and then were instructed to make an "online player profile" consisting of brief biography and photograph (to aid in the deception during the simulated-rejection task: Cyberball). After the profile was completed, participants provided saliva samples (Oragene-DISCOVER OGR-500 kits), were fitted with the Affectiva Q-Sensor (to index EDA), and then were seated in front of a 17-in. monitor to begin a series of three "on-line" ball games (i.e., simulated-rejection/acceptance task). Participants believed they were playing online against three other students at other locations on campus. Several techniques were employed to support the deception (e.g., player profiles, experimenter behaviors) and the effectiveness of the cover story was verified during debriefing. The rate of rejection/acceptance by the other players was standardized for all participants. In the first game, all participants were "tossed" the ball 25% of all the ball tosses (they were one of 4 "players") to produce a relatively neutral experience. In the second game, participants were systematically rejected and were only "tossed" the ball 7% of all tosses early in the period and then were "ignored" by other "players" to simulate rejection. In the final game, participants were systematically "accepted" and were "tossed" the ball 50% of all tosses throughout the game period to simulate acceptance.

Participants reported emotional responses after each of the three, 3-min games during a 2-min break and emotion-related facial behavior was recorded throughout using high-resolution video. Together these assessments yielded a total of six emotion response scores (3 reflecting negative emotion, 3 reflecting positive emotion) for each participant indexing emotions generated during the three games: neutral, rejection, and acceptance. Here, the neutral game served as baseline for each subject. In addition, in order to capture the ability to regulate emotion as the game changed from rejection to acceptance, responses to the rejection game were subtracted from responses to the acceptance by valence. As such, each participant also had one change score reflecting negative emotion regulation and one change score reflecting positive emotion regulation. Finally, each participant had a score for degree of sympathetic activation in each game indexed as EDA.

Manipulation Check

Significantly greater positive affect reported during positive emotion videos (*Alive*: $t(110) = -13.436$, $p < .001$; *Between Two Ferns*: $t(110) = -24.087$, $p < .001$) and greater negative affect reported during negative emotion videos (*Road to Guantanamo Bay*: $t(110) = 7.557$, $p < .001$; *The Champ*: $t(110) = -3.035$, $p < .003$) confirmed the efficacy of emotion inductions in Study 1. Likewise, in Study 2, participants reported significant increases in negative affect from warmup (baseline) to game 1 (rejection), $t(103) = -2.248$, $p < .03$, and decreases from Game 1 to Game 2 (acceptance), $t(103) = 4.413$, $p < .001$. In a similar fashion, positive affect significantly decreased from warmup (baseline) to Game 1 (rejection), $t(103) = 10.37$, $p < .001$, then increased from Game 1 to Game 2 (acceptance), $t(103) = -4.909$, $p < .001$. Finally, for Study 2, during the debriefing, we queried and confirmed that participants believed the deception that they were playing a game online with other participants, having no idea that the games were, in fact, a computer simulation. Indeed, the efficacy of Cyberball to elicit strong feelings of ostracism and rejection has been demonstrated repeatedly over the past decade, even when participants are informed prior to testing that the procedure is a computer simulation and does not involve any human component (Zadro, Williams, & Richardson, 2004).

Data Analytic Strategy

In both studies we first assessed patterns of negative and positive emotion regulation responses, specifically testing whether there were differences in the generation of emotion *within* contexts (i.e., reactivity) by variation in genotype. Next we examined change scores, focusing explicitly on the ability to regulate emotion from negative contexts to positive contexts (i.e., down-regulate negative emotion, up-regulate positive emotion) since prior work and considerable clinical theory would suggest that would be most consistent with both symptoms and vulnerability for emotional disorders (Kashdan & Rottenberg, 2010; Nolen-Hoeksema & Watkins, 2011). For each study, we used multivariate analyses when assessing emotion generation in order to control for shared variance across the many contexts, employing univariate follow-up tests, and univariate analyses when we examine each change score. For Study 2, we were able to control for responses to the neutral first game, adding additional rigor to those analyses. However, for both sets of analysis, we controlled for a number of key factors well-established as influencing emotional responses (e.g., gender, symptoms) in order to better isolate the influence of genotype. Based on prior research, we predicted that S′S′ individuals would respond with greater negative emotion to negative contexts. Moreover, we hypothesized that an S′S′ genotype would also be associated with less ability to regulate negative emotional responses when transitioning to positive contexts (resulting in lower change scores as well as higher negative emotion during positive contexts).

Results

Study 1

We examined demographic and symptom variables in relation to variations in 5-HTTLPR and the associated SNP rs25531, specifically S′S′ versus other alleles using either chi-square or *t* tests. There were no differences across groups by genetic variation (Table 1). Next, we examined differences in all emotion response indicators, controlling for factors well-established as influencing emotional responses (i.e., age, gender, race, ethnicity, depression) using a 2 (video type) × 2 (valence of emotion) × 2 (genotype: S′S′ vs. S′L/LL) analysis of covariance[3] (ANCOVA). There was a significant three-way interaction for Video × Valence × Genotype (Pillai's trace = .04, $F(1, 104) = 4.15$, $p < .05$). Although we did not find a main effect for genotype (i.e., that S′S′ participants had greater negative or positive emotion across videos), follow-up contrast tests revealed that there were significant differences in negative emotional responses during the positive film differentiating the S′S′ genotype from others (LS′ or LL). Specifically, the S′S′ genotype was associated with greater negative emotion during the positive films, $F(1, 103) = 3.89$, $p = .06$, partial eta^2 = .03, 95% confidence interval of the mean (CI): −.04, .93. In addition, univariate analysis (ANCOVA) of each change score revealed that S′S′ participants showed worse down-regulation of negative emotion, $F(1, 104) = 3.35$, $p < .07$; partial eta^2 = .03, 95% CI: −.04, 1.03, as well as a marginally lower increase (up-regulation) in positive emotion, $F(1, 104) = 2.73$, $p < .10$; partial eta^2 = .03, 95% CI: −1.12, .10, as participants transitioned from the negative films to the positive films. There were no significant multivariate effects for genotype when evaluating positive emotional responses generated across contexts—although there was a main effect of gender, such that males responded with greater positive emotion during the negative films. A graph of emotion responses by genotype demonstrates that this pattern of findings was largely consistent with our hypothesis (Figure 1).

Study 2

We examined demographic and symptom variables in relation to variations in the 5-HTTLPR and the SNP rs25531, specifically S′S′ versus other genotypes using either chi-square or *t* tests. As before, there were no differences across groups by genetic variation (see Table 1) and we examined differences in all emotion response indicators, controlling for factors well-established as influencing emotional responses (i.e., age, gender, race, ethnicity, depression) using a 2 (game type) × 2 (emotion valence) ×

[3] Assumptions of ANCOVA/MANCOVA were met, including equality of covariance matrices, and follow-up univariate tests employed Bonferroni corrections.

Table 1
Sample Characteristics by Variation in SLC6A4

	S′S′	S′L/LL	
	$n = 28$	$n = 83$	
Study 1: Demographic variables			
Age	$M = 21.32$ (7.46)	$M = 20.72$ (6.29)	$t(109) = -.42, p = .62$
Gender	18 Female	53 Female	$\chi^2 = .00$ $p = .97$
Race			$\chi^2 = 3.44$ $p = .33$
Caucasian	22	66	
Black/African American	2	11	
Asian	2	1	
Other	2	5	
Ethnicity	1 Hispanic/Latino	6 Hispanic/Latino	$\chi^2 = .47$ $p = .49$
Symptoms (CES-D)	$M = 12.04$ (6.92)	$M = 11.57$ (6.30)	$t(109) = .29, p = .47$
Standardized emotion response scores[a]			
Negative emotion in negative contexts	$M = -.10$ (1.47)	$M = -.07$ (1.28)	
Negative emotion in positive contexts	$M = .24$ (1.30)	$M = -.20$ (1.05)	
Positive emotion in negative contexts	$M = .14$ (1.38)	$M = -.33$ (.99)	
Positive emotion in positive contexts	$M = -.14$ (1.58)	$M = -.03$ (1.31)	
Negative emotion change score	$M = .25$ (1.32)	$M = -.26$ (1.24)	
Positive emotion change score	$M = .38$ (1.30)	$M = .17$ (1.47)	
	$n = 33$	$n = 71$	
Study 2: Demographic variables			
Age	$M = 21.18$ (6.30)	$M = 20.16$ (3.85)	$t(102) = -1.03, p < .31$
Gender	23 Female	40 Female	$\chi^2 = 1.68$ $p < .19$
Race			$\chi^2 = 3.07$ $p < .38$
Caucasian	27	64	
Black/African American	3	5	
Asian	2	2	
Other	1	0	
Ethnicity	1 Hispanic/Latino	3 Hispanic/Latino	$\chi^2 = .09$ $p < .77$
Symptoms (CES-D)	$M = 10.89$ (7.22)	$M = 11.66$ (6.86)	$t(102) = .53, p = .60$
Trait rejection sensitivity	$M = 8.12$ (3.43)	$M = 8.47$ (3.55)	$t(102) = .48, p = .63$
Standardized emotion response scores[a]			
Negative emotion in baseline game	$M = .27$ (1.69)	$M = -.16$ (1.27)	
Positive emotion in baseline game	$M = .57$ (1.99)	$M = -.14$ (1.45)	
Negative emotion in Game 1 (rejection)	$M = .15$ (1.56)	$M = -.05$ (1.26)	
Positive emotion in Game 1 (rejection)	$M = .67$ (1.99)	$M = -.24$ (1.26)	
Negative emotion in Game 2 (acceptance)	$M = .52$ (2.01)	$M = -.16$ (1.11)	
Positive emotion in Game 2 (acceptance)	$M = .54$ (1.72)	$M = -.14$ (1.36)	

Note. There were *no* significant differences by genotype for any demographic, symptom or trait variables in both studies.
[a] Standardized scores are aggregates of participant reports of emotion and observer-coded facial emotion.

2 (genotype: S′S′ vs. S′L/LL) repeated measures ANCOVA. Again there was a three-way interaction for Game Type × Valence × Group, although it was only marginally significant, Pillai's trace = .03, $F(1, 94) = 2.89$, $p < .09$. However, there were also significant multivariate effects for genotype, $F(1, 94) = 3.39$, $p < .07$, partial eta^2 = .04, and gender, $F(1, 94) = 10.47$, $p < .05$, partial eta^2 = .06. Follow-up contrast tests revealed significantly greater emotion (negative and positive) across contexts in the S′S′ group, $F(1, 93) = 5.43$, $p < .05$, partial eta^2 = .06, 95% CI: .05, .65, as well as significantly greater emotion in males across contexts, $F(1, 93) = 9.61$, $p < .01$, partial eta^2 = .09, 95% CI: −.74, −.16. Next, we conducted univariate analysis (ANCOVA) follow-up tests to examine differences in negative emotion generation in the rejection game (negative context) and acceptance game (positive context), controlling for responses to the neutral game. Consistent with Study 1, there was no difference in negative emotion by genotype for the negative context, but a significant difference during the positive context, such that individuals with the S′S′ genotype had significantly greater negative emotion during peer-acceptance, $F(1, 95) = 4.62$, $p < .05$; partial eta^2 = .05, 95% CI: .04, .93. In addition, there was a marginal difference in positive emotion during

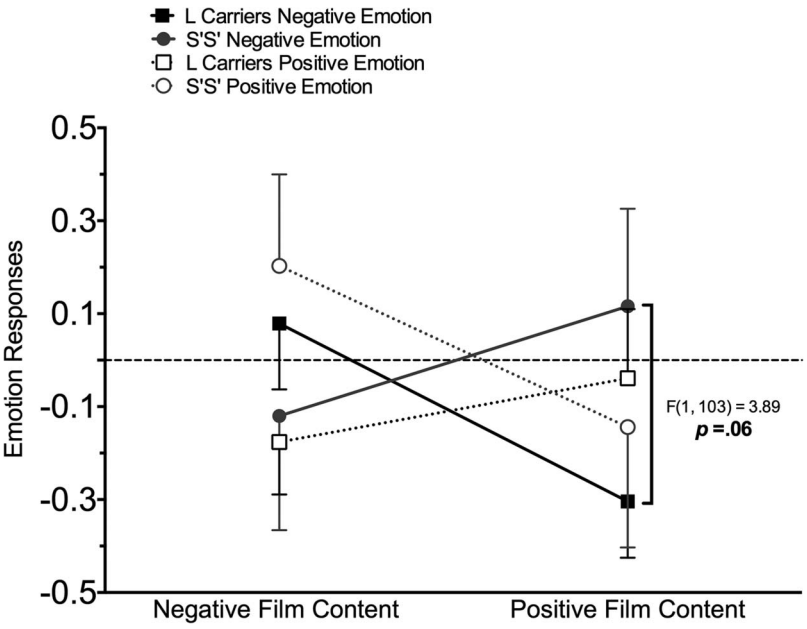

Figure 1. Emotion context in-sensitivity related to variation in SLC6A4 while viewing emotionally evocative films in Study 1. Data are presented as the mean ± *SEM*.

the negative context, such that individuals with the S′S′ genotype also had greater positive emotion during peer-rejection, $F(1, 95) = 3.40$, $p < .07$; partial eta^2 = .04, 95% CI: −.03, .88. In terms of emotion regulation, there was a difference in negative emotion regulation indicating that consistent with Study 1, S′S′ individuals had greater difficulty down-regulating negative emotion as they transitioned to a positive context, $F(1, 96) = 3.73$, $p < .06$; partial eta^2 = .04, 95% CI: −.01, .89. There were no other differences in emotion generation or regulation (Figure 2).

Finally, we also examined EDA by genotype using a repeated measures ANCOVA, controlling for factors that typically influence autonomic activity (age, sex, height, weight). We examined across trials of Cyberball (including the neutral warmup) and found a clear between-subjects effect for genotype and no interaction with game type. Specifically, carriers of the S′S′ alleles had significantly greater EDA across all games, $F(1, 89) = 7.07$, partial eta^2 = .07, $p < .01$, suggestive of greater sympathetic arousal in individuals with this genotype (Figure 3).

Discussion

Over the last two decades there has been increasing research attention focused on better understanding the link between common genetic mutations or polymorphisms and risk for psychiatric disease. This work has been based on considerable theory and compelling evidence suggesting the clear contribution of genetic differences to lifelong risk of psychiatric disease (cf., Hariri et al., 2005; Holmes, 2008). Most clearly, variation in SLC6A4 has been implicated across species with an increase in some early emotion processing biases and neuroanatomical differences that may contribute to increased risk for common emotional disorders such as depression or anxiety. In the current study we add to this literature by providing the first evidence of a 5-HTTLPR genotype-dependent influence on emotion regulatory responses evoked naturalistically in the lab. Specifically, across two studies we demonstrated that for individuals with two copies of the S′ allele there is a pattern of poor down-regulation of negative emotion, when the contextual parameters change to be explicitly positive. Poor

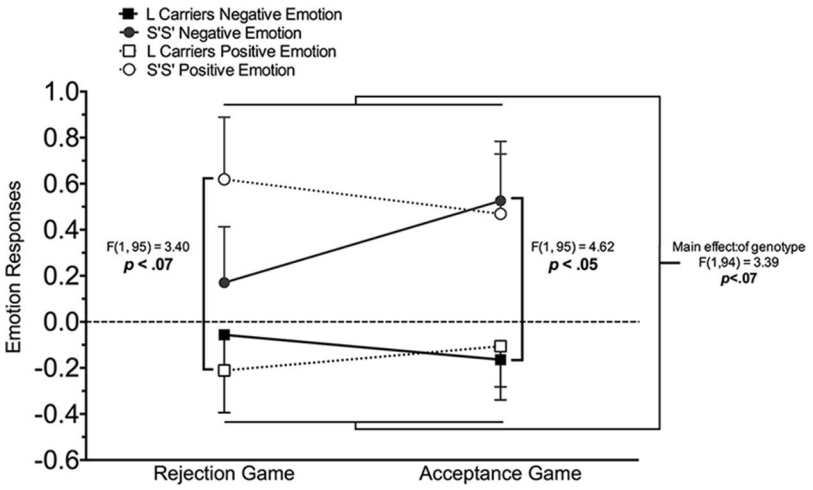

Figure 2. Emotion context in-sensitivity related to variation in SLC6A4 while experiencing simulated peer-rejection and acceptance in Study 2. Data are presented as the mean ± *SEM*.

regulation of negative emotion is implicated in most, if not all, emotional disorders (Gotlib & Joormann, 2010; Mathews & MacLeod, 2005) as well as risk-related clinical phenomena (e.g., rumination/worry; McLaughlin & Nolen-Hoeksema, 2011; Watkins, 2008). These data provide new evidence of how variation in 5-HTTLPR can influence clinically salient phenomena, in particular, emotion regulatory responses, and therefore offer a new target for future clinical investigation and even intervention.

Consistent with the majority of the extant literature on SLC6A4, we did also find evidence of greater negative emotional reactivity (i.e., higher negative emotion generated during negative contexts) in association with the S′ allele in Study 2. However, our data also suggested that the S′ allele predicted greater positive emotional responses as well as greater electrodermal activity across contexts. It may be that the emotion induction of peer-rejection in Study 2 was particularly salient for participants, thereby accounting for the difference in this finding across

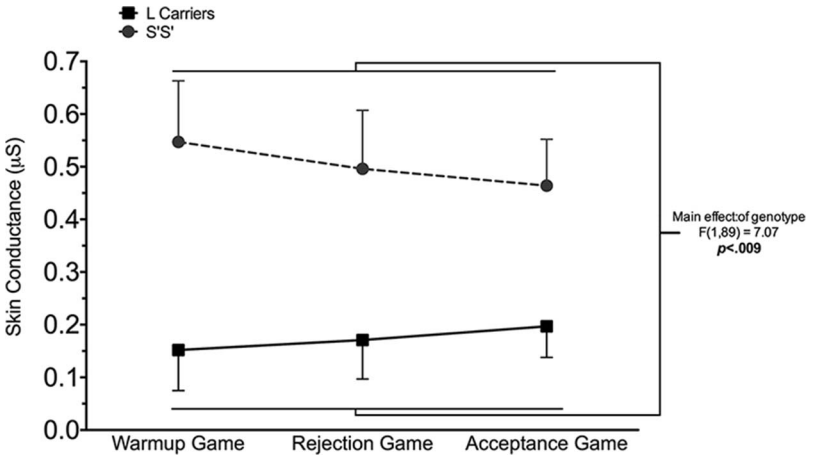

Figure 3. Increased sympathetic arousal related to variation in SLC6A4 while experiencing simulated peer-rejection and acceptance in Study 2. Data are presented as the mean ± *SEM*.

our two studies. In addition, there has been other research consistent with these findings (e.g., Crişan et al., 2009), suggesting that greater investigation of SLC6A4 in relation to multiple channels of emotion responsivity and multiple induction methods is warranted.

For the purposes of this investigation, we operated under a conservative a priori hypothesis that individuals homozygous for the S′ allele would exhibit impairments in regulation of negative emotions under changing emotional conditions. This is in line with findings of studies of the 5-HTTLPR using objective measures of genotype-dependent changes to environmental influences (Belsky & Pluess, 2009; Karg et al., 2011; Serretti et al., 2007). Similar studies measuring correlational physiological and/or emotional measures with respect to 5-HTTLPR genotype have also found strong effects in S′S′ populations compared with L carriers (Beevers et al., 2009; Gotlib et al., 2008; McCaffery et al., 2003). For those readers interested in emotional and physiological data across the three functional 5-HTTLPR genotypes, please refer to the supplementary table (Table S1).

Although our research is not the first to investigate SLC6A4 in relation to negative emotion, it is the first to demonstrate a clear tie to clinically salient deficits in emotion regulation indexed using a highly naturalistic and ecologically valid emotion regulation paradigm. Indeed, the focus of our assessment was decidedly downstream of most prior work which has typically indexed responses to *brief* exposure of negative stimuli. In contrast, in this investigation we sought to capture naturalistic responses to emotional content, more consistent with emotional experiences in daily life. As such, we indexed emotion responses as they spontaneously emerged over several minutes, multiple times, and focused on objective behavioral evidence (facial expressions; autonomic activity) as well as reports of experience. These indices are far later in the stream of emotion processing as compared to other commonly used assessments in prior research on 5-HTTLPR (e.g., indexing attentional bias, or response inhibition) and therefore provide a clearer link to the clinical meaning of genotypic variation, helping to fill what is still a relatively large gap in the literature.

Limitations

There were some limitations in this investigation. Because we assessed real-time emotional and physiological responses, sample sizes were constrained by the methods needed to analyze the evoked reactions to emotional manipulation (e.g., manually coding facial expressions to generate behavioral indices of emotion regulation, cleaning physiological measures, etc.). In turn, the benefit of these labor-intensive methods is that they result in more objective and valid indicators of naturalistic emotion regulation. Indeed, it is clear that linking genetic variation to salient clinical phenomena is an important and relatively untapped area of research. As such, future studies would benefit from including other ecologically valid methods including experience sampling as well as manipulations during within-lab assessments (e.g., induction of rumination vs. distraction).

Our statistical evaluations produced findings of sometimes marginal significance, likely attributable in part to the sample sizes for Studies 1 and 2. Future studies with larger numbers of healthy subjects, in addition to evaluation of clinical populations, will be important when replicating these findings. Larger sample sizes would also strengthen the power to evaluate heterozygous (LS′) individuals separately from homozygous S′S′ and LL groups instead of grouping the former with one of the latter, as we have done here and is frequently done in the literature (Serretti et al., 2007). Further complicating our analyses is the assumption that a single genetic difference between groups of individuals can be measured through distal processes such as facial emotion, electrodermal responsivity, and self-report of emotion. Had we collapsed our subject pool to use only the methods in Study 1 (or Study 2), we could have had a larger sample size and thus potentially observed stronger significance. However, we would argue that the strength of the evidence reported here is that we evaluated two separate cohorts responding to distinct yet highly valid emotion provocations to index one construct: naturalistic emotion regulation, in the context of a single genotypic difference. The results obtained from both studies can be perceived as a two-pronged approach to support our interpretation implicating the S′S′ genotype as being

associated with an impaired ability to adaptively process negative emotions.

Our subject pool consisted of an overwhelming majority of Caucasian individuals, preventing evaluation of race- or ethnicity-specific stratification of effects. This shortcoming could be addressed by replication of these methods in regions without a predominant Caucasian presence. An additional limitation was our focus on the 5-HTTLPR exclusively, rather than in relation to or in interaction with other genotypic variations within SLC6A4 or other related genes. The literature has focused almost exclusively on single gene associations when examining behavioral or other response outcomes, while it is increasingly clear that interactions occurring among and between systems are influenced by complex genotypic combinations. As with all genetic polymorphism association studies, it remains possible that when analyzing differences associated with 5-HTTLPR genotype we are unintentionally measuring the effects of an unidentified gene or polymorphism that is in linkage disequilibrium with the S′ allele.

Clinical Implications

These data have implications for the clinical understanding of risk and vulnerability afforded by variation in 5-HTTLPR. Indeed, although considerable prior work has suggested a link between the functional S′ allele and increased risk for emotional disorders, there is a paucity of data attempting to capture how this may actually manifest behaviorally. In particular, our data suggest consistently that individual carriers of two S′ alleles were less able to down-regulate negative emotions when contextual parameters became explicitly positive. This was evident even when controlling for factors such as depression symptoms and dispositional factors (i.e., rejection sensitivity) often demonstrated to predict similar patterns of emotional responding (Downey & Feldman, 1996; Gotlib & Joormann, 2010). Our data suggest a clear line of vulnerability to disorders in which generalized patterns of poor regulation of negative emotion are clearly evident (e.g., major depression, generalized anxiety disorder, generalized social phobia/social anxiety). Moreover, these data suggest that inhibition of negative emotion may be an important target for future investigation of treatments in clinical samples in which variation in 5-HTTLPR is known.

Conclusion

In this investigation we strove to better understand how variation in the 5-HTTLPR may contribute to increased risk for psychiatric disease by examining naturalistic emotion regulatory responses in two lab paradigms. In Study 1, participants were assessed while viewing four evocative films well-validated to elicit a range of strong negative or positive emotions. In Study 2, we employed a highly ecologically valid elicitor of negative emotion, ostracism (Williams, 2007). Across both studies our findings consistently suggested that a clear target for future clinical investigation may be the link between variation in the 5-HTTLPR and deficits in down-regulation of negative emotional responses. Specifically, we found that individuals who carried two copies of the S′ allele were significantly less able to down-regulate negative emotion when the context became explicitly positive. Moreover, in Study 2 during a series of socially challenging contexts, there was clear evidence of greater system-wide emotional reactivity. Given that both phenomena are consistently linked to affective disorders, these findings suggest key areas of vulnerability for S′ carriers, as well as potential targets for clinical intervention.

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