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Stressing over anxiety: A novel interaction of 5-HTTPLR genotype and anxiety-related phenotypes in older adults

Nia Fogelman, Anatoly Mikhailik, Anett Mueller-Alcazar[1], Kristin Bernard, Turhan Canli*

Department of Psychology, Stony Brook University, Stony Brook, NY, 11794-2500, USA

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ABSTRACT

Variation within the serotonin transporter gene-linked polymorphic region (5-HTTPLR) contributes to individual differences in trait neuroticism and increases risk for the development of psychopathology in the context of stressful life events. The underlying mechanisms may involve dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and the release of stress-related hormones. Yet, observed effects are small, possibly because they occur against the background of many other, mostly unknown, genetic and environmental variables. In this study, we removed much of the variance contributed by such background factors by including complex trait and behavioral measures in our analyses, to isolate the unique contributions of 5-HTTLPR genotype to cortisol baseline, reactivity, and recovery during the Trier Social Stress Test. We recruited 82 community-dwelling older adults (55 and older), an under-studied population, and measured salivary cortisol levels at baseline and following the TSST. As a comparison group we also recruited 88 younger adults (males only, 18–51 years old). Neuroticism, trait anxiety, perceived stress levels, and early childhood trauma experiences were measured using self-report questionnaires. An exploratory factor analysis revealed a latent anxiety trait. Cortisol baseline levels were significantly elevated in older adult S-allele carriers (but not in LL-homozygotes) who scored higher on the latent anxiety trait, relative to S-allele carriers. No such differences were found among younger adults, nor amongst measures obtained during the reactivity or recovery periods. These results highlight the utility of taking into account background variables that may otherwise obscure associations between genetic variables and endophenotypes.

1. Introduction

One of the seminal foundations of "molecular psychology" (Canli, 2015) was the discovery of a link between a common variation within the serotonin transporter gene (SCL6A4), the serotonin-transporter-linked polymorphic region (5-HTTLPR), and complex personality traits (Lesch et al., 1996). Specifically, the 5-HTTLPR contains a 43 BP insertion/deletion polymorphism in which the short ("S") allele is less transcribed than the long ("L") allele (Heils et al., 1996), and it is the S-allele that is associated with elevated trait neuroticism (Lesch et al., 1996). Later work identified an independent single nucleotide A/G polymorphism (SNP; rs25531); L-alleles that also carry the G-allele (denoted by "L_G") are considered functionally equivalent to the S-allele (Hu et al., 2006; Wendland et al., 2006). An entire literature of replication and extension studies has since emerged based on these findings, which have been confirmed in multiple (Munafo et al., 2009b; Schinka et al., 2004; Sen et al., 2004) but not all (Munafo et al., 2005) *meta*-analyses.

Later work investigated the moderating effects of 5-HTTLPR genotype on the link between stressful life events and psychopathology, starting with the discovery from a large-scale longitudinal study that presence of the S-allele, in conjunction with stressful life events, predicted depressive symptoms (Caspi et al., 2003). Similarly, participants who were homozygous for the S-allele and had at least one traumatic life event exhibited a 6.7 fold increase in the odds of Major Depressive Disorder (MDD), compared to the 2.1 fold increase in those with at least one traumatic life event and the S/L or L/L allelic type (Kendler et al., 2005). These gene-by-environment (GxE) interactions appear to extend beyond depression, as other studies showed similar relationships in dissociative disorders (Pieper et al., 2011), post-traumatic stress disorder (Pietrzak et al., 2013), and a broad set of psychopathologies (Vinberg et al., 2014). These GxE interactions have again been

* Corresponding author.
E-mail address: turhan.canli@stonybrook.edu (T. Canli).
[1] Now at: MSH Medical School Hamburg, University of Applied Science and Medical University, Hamburg, Germany.

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supported in multiple (Karg et al., 2011; Uher and McGuffin, 2008, 2010; van Ijzendoorn et al., 2012) but not all (Munafo et al., 2009a; Risch et al., 2009) reviews and *meta*-analyses.

One plausible pathway by which 5-HTTLPR genotype may impose a stress-related toll is via the hypothalamic-pituitary-adrenal (HPA) axis and the release of stress-related hormones that can place an allostatic load on the body (McEwen, 1998). Indeed, several studies reported differential cortisol baseline or reactivity levels as a function of 5-HTTLPR genotype. For example, girls exposed to an acute laboratory stressor (backwards counting, followed by a social competency interview) who were homozygous for the S-allele exhibited significantly stronger cortisol reactivity, compared to girls who carried one or two copies of the L-allele (Gotlib et al., 2008). Differential levels of the cortisol awakening response as a function of 5-HTTLPR genotype have also been reported (Wust et al., 2009). A recent *meta*-analysis of the literature concluded that there was a small but significant association between 5-HTTLPR genotype and HPA-axis reactivity to acute psychosocial stress (Miller et al., 2013). However, this analysis also highlighted the need for additional studies, particularly in understudied populations such as older subjects, for which only one publication was available (Mueller et al., 2011).

In this study, we examined baseline cortisol and cortisol reactivity/recovery to the Trier Social Stress Test (Kirschbaum et al., 1993) in two samples: a cohort of older (age 55 and over) adults, and a cohort of younger adults (18–51 years old). Based on an S-allele dominant model, we aimed to test whether presence of the S-allele (based on the functionally defined triallelic classification scheme: S, L_A, L_G) predicted baseline cortisol or cortisol response to an acute stressor, and whether the impact of 5-HTTLPR genotype on cortisol was further moderated by early life stress, or by trait-related or chronic levels of negative affect.

2. Materials and methods

2.1. Participants

From an initial cohort of 94 adults aged 55 and older who were recruited from Stony Brook and the surrounding area via flyers and online postings, data from 82 older adults (M_{age} = 63.46, SD_{age} = 7.15; 46 women) were used in this study (data from 12 older adults were not used because they were run in the morning hours when cortisol levels are highly variable). Data from a subset of individuals were previously reported in the context of a study on 5-HTTLPR gene methylation (Duman and Canli, 2015). The ethnic distribution included Caucasian (91.5%), Asian (3.7%), Hispanic (3.7%), and African American (1.2%). A second cohort of 88 younger male adults was also recruited (M_{age} = 24.07, SD_{age} = 7.74). This group was almost exclusively Caucasian with only 1.1% Asian and 3.4% identifying with multiple races, and all subjects were run midday (1100h–1500 h) or in the evening (1500h–1900 h). Upon calling in, potential participants were informed that during the 4-h experiment they would provide blood and saliva samples and complete questionnaires, a life history interview and a mild stress task. Exclusion criteria included: being a smoker, experiencing substance or alcohol abuse, having a psychiatric diagnosis, taking psychiatric or hormonal medication, being diabetic, medication for cardiovascular disease, and currently being under immense stress. Additionally, all female subjects were post-menopausal; this condition addresses potential concerns regarding the effects of menstrual cycle status on cortisol measurements (Kirschbaum et al., 1999). The Committee on Research Involving Human Subjects (CORIHS) of Stony Brook University approved this research.

2.2. Measures

2.2.1. Questionnaires

Participants completed a number of questionnaires to assess for personality, childhood trauma and stress. We measured Neuroticism as a subscale of the NEO-FFI (Costa and McCrae, 1992). Sample questions included items such as *I often feel inferior to others* and *I am not a worrier* (reverse coded). Questions were answered on a 5 point Likert scale from "strongly disagree" to "strongly agree." Scores were then scaled into T scores based on normative samples in accordance with the professional manual to make them comparable across men and women (Costa and McCrae, 1992). To assess trait anxiety, participants answered the State Trait Anxiety Inventory (STAI; (Spielberger, 1983) about how much they generally agree with 20 statements. Example statements include *I am happy* and *I feel secure*, and were assessed on a 4 point Likert scale ranging from "almost never" to "almost always." Early life stress was measured using the Childhood Trauma Questionnaire (CTQ; (Bernstein et al., 1994). The sum score of this questionnaire considers emotional, sexual and physical abuse. Questions include items such as *I didn't have enough to eat* and *I felt someone in my family hated me*. The 5 point Likert scale offered options from "never true" to "very often true."

Stress was assessed with two measures. One was the Perceived Stress Scale (PSS;(Cohen et al., 1983), using 10 items to assess feelings on stress levels over the past month. It includes items such as *in the last month, how often have you found that you could not cope with all the things that you had to do*?, and, *in the last month, how often have you been able to control irritations in your life?* Participants answered using a 5 point Likert scale ranging from "never" to "very often." The second measure was the Trier Inventory for Chronic Stress Scale (TICS-CSSS; (Schulz and Schlotz, 1999)) and participants rated the frequency of various stressful experiences over the past 3 months from "never" to "very often." Items included *I worry that something unpleasant will happen* and *I experience having to do too much*.

2.2.2. 5-HTTLPR and rs25531 genotyping

Participants provided one blood sample. Peripheral blood mononuclear cells were extracted and stored in a −80 °C freezer until analysis. We investigated a common length polymorphism due to an insertion/deletion (indel) in the promoter region of the 5-HTTLPR gene. We independently also genotyped for a second A/G Single Nucleotide Polymorphism (SNP) rs25531 (Wendland et al., 2006). Because there were no 5-HTTLPR short-allele carriers who also carried the rs25531 G-allele, we treated the sample as functionally tri-allelic (S_A, L_A or L_G), with L_G treated as functionally equivalent to S.

5-HTTLPR classification was determined through amplification by the polymerase chain reaction (PCR) with the following primers: LPR_L 5′-GGGGAGATCCTGGGAGAGGT-3′, LPR_R 5′-CGCTCTGAATGCCAGCACCTA-3′ and HotStarTaq Plus Master Mix Kit (Qiagen). These primers help to generate DNA fragments of 215 bp (S-allele) and 258 bp (L allele). PCR was carried out with 500 nM of each, forward and reverse primers and 20 ng of template in a 20 μl total reaction volume in Mastercycler PCR device (Eppendorf, Germany). The cycling conditions included heat activation at 95 °C for 4 min, 41 cycles of 95 °C for 20 s, 68 °C for 20 s, and 72 °C for 20 s, followed by a final 2 min extension at 72 °C and hold at 4 °C. PCR products were run in 2% agarose gels stained with ethidium bromide and visualized using an InGenius gel documentation system (Syngene). Next, A/G SNP rs25531 status was determined, 7 μl of the 5-HTTLPRLPR PCR products were digested with 8 Units of MspI restriction enzyme (New England Biolabs, MA) for 2 h at 37 °C followed by an inactivation step of 20 min at 65 °C. Digestion