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The serotonin transporter-linked polymorphic region (5-HTTLPR) polymorphism, stress, and depression

Christina Di Iorio, Kimberly Johnson, Daniel Sheinbein, Samantha Kahn, Patrick England, Ryan Bogdan

BRAIN Lab, Psychological and Brain Sciences, Washington University in St. Louis, St. Louis, MO 63130

Correspondence: Christina Di Iorio (crdiiorio@wustl.edu) and Ryan Bogdan (rbogdan@wustl.edu; BRAIN Lab CB 1125 Psychological and Brain Sciences, Washington University in St. Louis, St. Louis, MO 63130

Abstract

Supportive of diathesis-stress models of depression, twin research first documented that broad genetic vulnerability to depression (i.e., an unaffected twin whose co-twin has a history of depression) potentiates the depressogenic effects of stress. In 2003, Caspi and colleagues provided evidence of a molecular genetic x stress interaction predicting depression when they found that the short 5-HTTLPR allele was associated with greater risk for stress-related depression. This highly influential study inspired numerous replication and extension attempts that have produced conflicting findings, with even meta-analyses reaching opposing conclusions. Neuroscience evidence suggests that the 5-HTTLPR polymorphism may confer vulnerability to depression through its association with amygdala function and early life exposure to heightened serotonin levels. Given mixed evidence of the relationship between 5-HTTLPR and stress-related depression as well as putative intermediate phenotypes, further empirical investigation is warranted.

Keywords: serotonin transporter, 5-HTTLPR, stress, depression, amygdala, serotonin, Caspi, G x E, gene by environment, development

Introduction

Diathesis stress theories postulate that genetic vulnerability promotes the development of depression in the context of stress. The intuitive appeal of this theory was supported by twin research by Kendler and colleagues (Kendler et al., 1995) showing that risk for depression was substantially elevated among individuals whose co-twin had depression, but only if they themselves were recently exposed to a severe stressful life event. However, it was not until 2003 that molecular genetic variation was first associated with vulnerability to the depressogenic effects of stress (Caspi et al., 2003). Specifically, Caspi and colleagues reported evidence that the short allele of the serotonin transporter-linked polymorphic region (5-HTTLPR) polymorphism, which had been previously linked to reduced serotonin transporter expression as well as putative intermediate depressive phenotypes (Hariri et al., 2002; Heils et al., 1996; Lesch et al., 1996), confers vulnerability to stress-related depression. This initial report of a gene x environment (GxE) interaction spurred multiple subsequent replication and extension attempts which have collectively produced conflicting findings with even meta-analyses reaching opposing conclusions (Duncan & Keller, 2011; Karg, Burmeister, Shedden, & Sen, 2011; Munafo, Durrant, Lewis, & Flint, 2009; Risch et al., 2009; Sharpley, Palanisamy, Glyde, Dillingham, & Agnew, 2014; Taylor & Munafo, 2016); **Table 1**). These opposing results have led to great controversy within the literature, with divergent interpretations (Caspi, Hariri, Holmes, Uher, & Moffitt, 2010; Duncan & Keller, 2011) highlighting the need for further study and collaborative meta-analyses (Moffitt & Caspi, 2014; Taylor & Munafo, 2016). Here, we briefly introduce the 5-HTTLPR polymorphism before summarizing the current literature on the 5-HTTLPR polymorphism and depression and exploring potential neural mechanisms through which these associations may emerge.

The 5-HTTLPR Polymorphism

The 5-HTTLPR polymorphism resides within the promoter region of the serotonin transporter gene (*SLC6A4*) (Lesch et al., 1996). The serotonin transporter (5-HTT) is a reuptake protein that regulates the duration and magnitude of synaptic serotonin. It is the target of common antidepressants and anxiolytics, such as serotonin specific reuptake inhibitors (SSRIs), which block the transporter resulting in increased synaptic serotonin and signaling. The 5-HTTLPR polymorphism is a 43(initially reported as 44)-base pair imperfect repeat sequence in a GC-rich repetitive sequence that is located approximately one kb upstream of the 5-HTT gene transcription initiation site (Wendland, Martin, Kruse, Lesch, & Murphy, 2006). There are two commonly occurring alleles within this polymorphism, the short (s; 14 repeat) and long (l; 16 repeat) repeat, resulting in 3 genotype groups (s/s, s/l, l/l), although uncommon extra long repeats have been reported in East Asian and some African samples (Goldman, Glei, Lin, & Weinstein, 2010; Nakamura, Ueno, Sano, & Tanabe, 2000). 5-HTTLPR allelic frequencies differ according to ancestral origin; relative to European samples, the frequency of the short allele is higher in Asian and lower in African populations with evidence that some phenotypic associations may be

dependent upon ancestral origin (Murphy, Maile, & Vogt, 2013). Notably, a common orthologue of the 5-HTTLPR in rhesus macaques enables cross species investigation (Caspi et al., 2010).

The 5-HTTLPR short allele has been associated with relatively less efficient transcription of the 5-HTT, resulting in less 5-HT uptake and elevated levels of synaptic 5-HT relative to the long allele (Heils et al., 1996; Lesch et al., 1996). In close proximity to the 5-HTTLPR polymorphism are two single nucleotide polymorphisms, rs25531 (G/A) and rs25532 (C/T), that moderate the influence of the 5-HTTLPR polymorphism on *SLC6A4* expression (Murphy et al., 2013). When the rs25531 G allele occurs alongside the 5-HTTLPR long allele, there is relatively less *SLC6A4* expression compared to when the rs25531 A allele is present (Hu et al., 2006). As a consequence, the presence of the G allele with the 5-HTTLPR long allele may render the long allele functionally similar to the short allele, leading many studies to recode alleles according to high and low expression with the 5-HTTLPR long and rs25531 G allele combination being grouped with the short allele as low expressing alleles. For rs25532, the T allele is associated with reduced *SLC6A4* expression, with a greater relative reduction when alongside the long relative to short allele (Wendland et al., 2008). While studies are more frequently including rs25531 in 5-HTTLPR investigations, the lack of consideration of rs25531 and rs25532 as well as other functional polymorphisms within *SLC6A4* may, at least in part, contribute to the mixed 5-HTTLPR psychiatric and neuroscience literature (Murphy et al., 2013).

5-HTTLPR Associations with Depression

The 5-HTTLPR polymorphism is among the most frequently studied genetic polymorphisms within psychiatry and neuroscience (Caspi et al., 2010). Notably, the vast majority of this research has been conducted in samples with European ancestral origins, leaving the generalizability of these findings across populations uncertain. Lesch and colleagues were the first to link this polymorphism to a psychiatric phenotype when they observed that the short allele was associated with elevated neuroticism (Lesch et al., 1996). This association has been replicated in some studies, with meta-analyses supporting evidence of a small but significant positive association between the short 5-HTTLPR allele and neuroticism, but not other anxiety-related measures (Munafo, Freimer, et al., 2009; Schinka, Busch, & Robichaux-Keene, 2004; Sen, Burmeister, & Ghosh, 2004). Further, similar associations with anxiety-like behavior have been observed with an orthologous variant in rhesus macaques (Caspi et al., 2010).

In a 2003 study that captured the attention of researchers and the general public alike, Capsi and colleagues reported evidence that the 5-HTTLPR polymorphism moderates the depressogenic effects of stress (Caspi et al., 2003). Specifically, these authors found that the short allele, which is associated with less 5-HTT expression and consequently less 5-HT reuptake and elevated synaptic 5-HT, was associated with greater rates of depression, but only among individuals who were exposed to adversity early in life or who experienced stressful life events as adults. The observation of a molecular GxE interaction predicting depression built upon existing twin studies (Kendler et al., 1995) and generated excitement that molecular GxE interaction studies may provide unique etiologic insight into psychopathology, and resulted in a host of subsequent

replication and extension studies. When these studies did not universally replicate the original report and subsequent meta-analyses reached opposing conclusions (**Table 1**), excitement was replaced by sober realizations that the effect was likely smaller than originally anticipated or potentially a false-positive association that has been perpetuated by publication and citation biases (de Vries, Roest, Franzen, Munafo, & Bastiaansen, 2016; Duncan & Keller, 2011).

Differences in meta-analytic approaches such as study inclusion and analytic procedures may have resulted in divergent outcomes (**Table 1;** (Taylor & Munafo, 2016)). Meta-analyses that only included studies which closely approximated the methodology of the original report (Caspi et al., 2003) found null effects (Munafo, Durrant, et al., 2009; Risch et al., 2009), while later meta-analyses that adopted broader criteria allowing for more studies to be included have reported evidence of a 5-HTTLPR x stress interaction predicting depression or related phenotypes (Karg et al., 2011; Sharpley et al., 2014) but see also (Taylor & Munafo, 2016). Meta analyses focusing on specific medical populations have produced mixed results (Mak, Kong, Mak, Sharma, & Ho, 2013; Suppli et al., 2015).

Inconsistent results across meta-analyses may also result from how variability in study design among included studies is modeled. The two meta-analyses reporting evidence that the 5-HTTLPR x stress interaction does predict depression conducted further analyses stratified by stress assessment and type of stressor (Karg et al., 2011; Sharpley et al., 2014). These found that all methods of assessment (e.g., objective measure, interview, self-report) produced significant effects but that objective and interview-based assessments of stress produced the most robust results (Karg et al., 2011; Sharpley et al., 2014) and that self-report measures, which are often used in larger studies with less deep phenotyping, were not robust to individual study exclusion within the meta-analysis (Karg et al., 2011). The explanation of this difference could be attributable to more refined phenotyping leading to more accurate assessments of stress exposure that may enhance real associations (Moffitt & Caspi, 2014) but could also be attributable to the fact that such deep phenotyping is often present in studies of smaller samples, which are inherently more prone to produce false positive and negative results.

The type of and timing of stress exposure appears to moderate the magnitude of the 5-HTTLPR x stress interaction predicting depression. For example, Karg et al. 2011, found that the 5-HTTLPR x stress interaction was much larger when considering childhood maltreatment and that this effect was robust to the exclusion of any single study, which was not the case for stressful life events predominantly assessed during adulthood. The larger effect of childhood stress in the 5-HTTLPR x stress interaction may be attributable to the robust effects of early life stress on depression, potential neurodevelopmental vulnerability (Ansorge, Zhou, Lira, Hen, & Gingrich, 2004; Bogdan, Agrawal, Gaffrey, Tillman, & Luby, 2014), temporal plausibility (Moffitt & Caspi, 2014), and the reduced association between subsequent depressive episodes and stressful life event exposure (Kendler & Gardner, 2016). With regard to the last point, the empirically-supported kindling theory of depression (Post, 1992) (Kendler & Gardner, 2016), suggests that initial episodes of depression are strongly predicted by the occurrence of stressful life events, while later episodes are predicted by prior episodes of depression but not major stressful life events. Implicit

in this hypothesis is that depressive episodes result in biological and/or psychological scars that set the stage for future episodes (i.e., these individuals are kindled). Importantly, however, stress may not be entirely unpaired from subsequent episodes, but the extent of stress needed to evoke depression may be diminished. More specifically, evidence suggests that minor stressors, such as daily hassles, which would not be detected in many assessments of stressful life events may promote a depressed episode in a kindled individual (Monroe & Harkness, 2005). As such, it is plausible that kindling may also moderate the 5-HTTLPR x stress interaction predicting depression. This is supported by evidence that the 5-HTTLPR x stress interaction is most robust when considering early life stress (Bogdan et al., 2014; Karg et al., 2011) as well as evidence that low level stressors during adulthood are most predictive of depression in the context of the 5-HTTLPR genotype x stress interaction (Kendler, Kuhn, Vittum, Prescott, & Riley, 2005). However, the possibility that kindling may influence the 5-HTTLPR x stress interaction predicting depression remains speculative as kindling has not been formally tested in the context of a longitudinal design and 5-HTTLRP x stress interaction.

One aspect of the 5-HTTLPR, stress, and depression literature that is puzzling is the directionality of association in studies reporting positive results. The short allele, which is associated with stress-related depression in some studies, is associated with less *SLC6A4* expression. Because, less 5-HTT availability is semi-analogous to the effects of common treatments for depression (e.g., SSRIs), the directionality of results may appear counterintuitive. However, converging work from non-human animal and human studies suggests that increases in 5-HT conferred by the short allele may result in latter depression due to elevated levels of serotonin experienced during early life and neurodevelopment. In a non-human animal model, rodents treated with SSRIs shortly after birth (equivalent to the third trimester of pregnancy in humans) behave normally as pups, but display depressive- and anxiety-like behavior later as adults (Ansorge et al., 2004). In a human cohort study using data from the Finnish national registry found that prenatal exposure to SSRIs was associated with a three- to four-fold risk of depression during adolescence relative to offspring born of mothers with psychiatric disorders who discontinued SSRI use during pregnancy and mothers with psychiatric disorders who were not taking medication (Malm et al., 2016). Collectively, these data suggest that unlike the antidepressant effects of increasing synaptic serotonin by blocking the 5-HTT in adults, that elevations of serotonin during prenatal development, and potentially into childhood, may increase risk for depression during adolescence and/or adulthood. Taken together, these findings suggest that the 5-HTTLPR short allele, which is associated with reduced 5-HTT efficiency and greater synaptic 5-HT levels, may increase risk for depression through exposure to elevated serotonin throughout development.

5-HTTLPR: Potential Neural Mechanisms Underlying Associations with Stress-related Depression

Amygdala and Neuroticism

Imaging genetics is a research strategy that examines associations between both genetic and epigenetic variation and variability in brain structure, function, and connectivity, as well as risk for psychopathology (R Bogdan et al., In press). Imaging genetics provide a unique approach to mechanistically relate differences in 5-HTTLPR genotype to neural systems that mediate cognition, emotion, and behavior in health and disease (Hariri & Holmes, 2006). Most commonly, 5-HTTLPR imaging genetics studies have investigated amygdala responsivity to threatening information. Broadly, the amygdala and its connections are necessary for recognizing possible threat in the environment and then generating and regulating physiologic and behavioral reactions. Elevated amygdala response to threat is a hallmark of various forms of psychopathology and in particular stress-related disorder (Bogdan, Pagliaccio, Baranger, & Hariri, 2016). Amygdala function is regulated by serotonin (Holmes & Hariri, 2003) and Hariri and colleagues (Hariri et al., 2002) linked the short allele to elevated amygdala reactivity in one of the first imaging genetics studies. This finding has not been consistently replicated across studies, with meta-analyses concluding that a small effect may be present (Munafo, Brown, & Hariri, 2008; S. E. Murphy et al., 2013), but see also (Bastiaansen et al., 2014). As such, heightened amygdala reactivity associated with the short allele remains a plausible mechanism, among many (e.g., hypothalamic-pituitary adrenal axis function; (Gotlib, Joormann, Minor, & Hallmayer, 2008)) through which the 5-HTTLPR polymorphism may confer vulnerability to the depressogenic effects of stress. Indeed, recent evidence that elevated amygdala reactivity prospectively predicts elevated depression symptoms following stressful life event exposure, provides support for this speculation (Swartz, Knodt, Radtke, & Hariri, 2015). A related interpretation is that given the links between amygdala reactivity and neuroticism (Cunningham, Arbuckle, Jahn, Mowrer, & Abduljalil, 2010), it is plausible that elevated amygdala reactivity among short allele carriers contributes to neuroticism, which itself moderates the impact of stressful life events on the development of depression (Kendler, Kuhn, & Prescott, 2004). Future adequately powered prospective longitudinal studies employing structural equation models alongside convergent non-human animal models, are needed to evaluate these putative mechanisms (Bogdan et al., 2016).

Neural Development

As alluded to earlier, 5-HTTLPR genotype may confer vulnerability to stress-related depression due to the neurodevelopmental consequences of reduced 5-HTT function. First, as mentioned above, pharmacologic blockade of the 5-HTT during early development in rodents (perinatal) and humans (prenatal) is associated with elevated anxiety and depression in adolescence and adulthood (Ansorge et al., 2004; Malm et al., 2016). Second, human neuroimaging studies suggest that the associations between 5-HTTLPR genotype and amygdala function is not related to current 5-HTT

availability as measured with positron emission tomography, but by brain structure; this raises the possibility that differences conferred by 5-HTT genotype arise from indirect neurodevelopmental consequences on brain structure as opposed to its direct acute effects on serotonin transporter function (Kobiella et al., 2011). Lastly, there is the possibility that both 5-HTTLPR and early life stress may both result in downstream consequences associated with elevated 5-HT activity. For instance, both 5-HTTLPR genotype and early life stress are associated with diminished 5-HT1A receptor binding suggesting a potential shared molecular pathway with additive influences (Spinelli et al., 2010). Further, this reduced 5-HT1A receptor binding is associated with differences in neurodevelopment and may confer vulnerability to depression and anxiety through disrupted prefrontal regulation of the amygdala and other subcortical structures (Pezawas et al., 2005).

Conclusions

The initial excitement generated by the Caspi et al., 2003 report that variation in the common 5-HTTLPR polymorphism moderates the depressogenic effects of stress have been tempered by replication studies and meta-analyses that have reached opposing conclusions. What is presently clear is that whether 5-HTTLPR genotype increases the risk for stress-related depression remains an empirical question that will be best informed by large longitudinal studies with deep phenotyping. In addition to conducting meta-analyses of existing studies that model between study variability, it is important for further research to evaluate possible reasons that may contribute to this mixed literature, such as the timing of stress exposure and whether kindling may reduce the 5-HTTLPR x stress interaction. Lastly, given accumulating evidence of epigenetic variation within *SLC6A4* associated with depression –related phenotypes (Nikolova et al., 2014; Swartz, Hariri, & Williamson, 2016), it will be important to not only consider genetic variation across the serotonergic system and related systems, but also epigenetic signatures.

Table 1: Meta-analyses of the 5-HTTLPR x Stress Interaction and Depression			
Meta-Analysis	**Data Coding**	**Meta-analytic Method**	**Conclusion**
(Munafo, Durrant, et al., 2009) 15 studies 11,158 participants	**Depression:** Depression diagnoses and self-reported depression symptoms and severity **Stress:** Presence (1) or absence (0) of stressful life event. Also tested 2 or more stressful life events relative to 0-1. Stress included lifetime trauma exposure, maltreatment during childhood. **Genotype: S**hort allele dominance, i.e., S carriers (S/S, or S/L) vs. L homozygotes (L/L)	Evaluated main effects of 5-HTTLPR and stress as well as their interaction using fixed- effect model pooling odds ratios (ORs). Random effect models implemented when significant association observed in the context of between study heterogeneity.	No evidence of GxE, OR: 1.16, 95% CI: 0.89 – 1.49. Results did not differ with random effects models or with different SLE coding.
(Risch et al., 2009) 14 studies 14,250 participants	**Depression:** Depression diagnosis (interview-based diagnosis or >85% on a measure of depression symptoms) **Stress:** Stressful life events (i.e., 0, 1, 2, ≥3). **Genotype:** Number of S alleles (additive): 0 (L/L), 1 (S/L), 2 (S/S). Also compared models of S dominance (i.e., S carriers vs Long Homozygotes) or recessive (i.e., S homozygotes vs Long Carriers).	Evaluated main effects of 5-HTTLPR and stress as well as their interaction using random- effects model pooling ORs. Also evaluated presence of gene-environment correlation (rGE). Analyses conducted with sexes combined (14 studies) and separated (10 studies).	No evidence of GxE, OR: 1.01, 95% CI: 0.94 – 1.10. Results did not differ when sexes were considered separately. Findings comparable in dominant and recessive models.
(Karg et al., 2011) 54 studies 40,749 participants	**Depression:** Depression diagnosis and self-reported depression symptoms and severity **Stress:** Childhood maltreatment, specific medical conditions, and SLE **Genotype:** As coded in original study	P values extracted from effects reported in each article (using a variety of genotype coding, stress measures, and depression measures). Analyses conducted across all forms of stress reported and stratified by stressor type (i.e., childhood maltreatment, specific medical conditions, and SLE), as well as method of stress assessment	Significant GxE: $p < 0.00002$. Short allele associated with greater depression in context of stress exposure. Robust to the exclusion of any single study. Stratified analyses by stressor type: childhood maltreatment $p = 0.00007$), specific medical group, $p = 0.0004$, SLE $p = 0.03$. Robust to the exclusion of any single study in the childhood maltreatment and medical group analyses but not the SLE analyses. Stratified analyses by stress assessment: Objective measure: $p = 0.000003$, interview: $p = 0.0002$, self-report questionnaire: $p = 0.042$. Robust to the

		(i.e., objective, interview, and self-report).	exclusion of any single study in the objective measure and interview analyses but not the self-report questionnaire analyses.
(Mak et al., 2013) 4 studies 642 participants	**Depression:** Assessed clinically by structured psychiatric diagnostic interview; either post-stress depression or no depression after stroke **Stress:** Stroke diagnosis confirmed by imaging studies or medical record **Genotype:** Short allele dominance, i.e., S carriers (S/S or S/L) vs L homozygotes	Evaluated the relationship between 5-HTTLPR genotype variations and risk of developing post-stroke depression (PSD). Random-effects pooled OR.	SS genotype associated with greater post stroke depression, OR: 2.05, 95% CI 1.41 to 2.98, z = 3.79, p<0.001).
(Sharpley et al., 2014) 81 studies 55,269 participants	**Depression:** Assessed by either self-report depression scale or clinical interview **Stress**: Stress included illness, childhood adversity, and recent life stress. **Genotype:** Short allele dominance, i.e., S carriers (S/S or S/L) vs L homozygotes.	P values extracted from each article. Articles were classified according to whether the studies supported the relationship between the *s* allele, stress, and depression or whether the studies did not support the relationship. Stratified by research design, stressor type, and type of stressor assessment.	Significant GxE, p = .0000009. Short allele associated with increased depression in the context of stress exposure. Robust to the exclusion of any single study. Stratified stress assessment: Objective method: p = 0.00000100, Interview: p = 0.00002486, Self-report questionnaire: p = 0.00152065. Robust to exclusion of any single study. Stratified by type of stressor: Medical condition: p = 0.00029, Childhood adversity: p= 0.00026011, Stressful life events: p = 0.00061359. Robust to exclusion of any single study. Stratified by research design: Exposed-only: p = 0.002, Longitudinal: p = 0.0026, Case-control: p = 0.0061, Cross-sectional: p = 0.003. Exposed-only association is nonsignificant when one study removed. 21 of 81 studies (nearly 26%) did not support association between *s* allele, stress, and depression.

(Suppli et al., 2015) 5 studies 1,484 participants (33 head and neck cancer patients and 642 breast cancer patients, 806 colorectal cancer patients)	**Biallelic genotype: S**hort allele dominance: SS, SL, and LL as comparison **Depression:** users and non-users of antidepressants	Evaluated the association between 5-HTTLPR and depression in cancer patients. Calculated odds ratios using numbers of antidepressant users and non-users in biallelic genotype groups. ORs unadjusted and inverse variance weighting for pooling. Computed random-effects models	No evidence that 5-HTTLPR is associated with depression among cancer patients (SL genotype: OR: 1.44, 95% CI: 0.78-2.65; SS genotype (OR: 1.05, 95% CI: 0.71-1.57).

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