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Stress exposure and psychopathology alter methylation of the serotonin receptor 2A (*HTR2A*) gene in preschoolers

STEPHANIE H. PARADE,[a,b] ANDREW M. NOVICK,[a,c] JUSTIN PARENT,[a,b,d] RONALD SEIFER,[a,b] SAMANTHA J. KLAVER,[b] CARMEN J. MARSIT,[e] ASI POLLY GOBIN,[c] BAO-ZHU YANG,[f] AND AUDREY R. TYRKA[a,c]

[a]*Brown University Alpert Medical School;* [b]*E. P. Bradley Hospital;* [c]*Butler Hospital;* [d]*Florida International University;* [e]*Emory University; and* [f]*Yale University School of Medicine*

Abstract

Serotonin signaling pathways play a key role in brain development, stress reactivity, and mental health. Epigenetic alterations in the serotonin system may underlie the effect of early life stress on psychopathology. The current study examined methylation of the serotonin receptor 2A (*HTR2A*) gene in a sample of 228 children including 119 with child welfare documentation of moderate to severe maltreatment within the last 6 months. Child protection records, semistructured interviews in the home, and parent reports were used to assess child stress exposure, psychiatric symptoms, and behavior. The *HTR2A* genotype and methylation of *HTR2A* were measured at two CpG sites (–1420 and –1224) from saliva DNA. *HTR2A* genotype was associated with *HTR2A* methylation at both CpG sites. *HTR2A* genotype also moderated associations of contextual stress exposure and *HTR2A* methylation at site –1420. Contextual stress was positively associated with –1420 methylation among A homozygotes, but negatively associated with –1420 methylation among G homozygotes. Posttraumatic stress disorder and major depressive disorder symptoms were negatively associated with methylation at –1420, but positively associated with methylation at –1224. Results support the view that the serotonin system is sensitive to stress exposure and psychopathology, and *HTR2A* methylation may be a mechanism by which early adversity is biologically encoded.

Childhood stress exposure including maltreatment and exposure to other contextual stressors associated with poverty confer risk for physical and mental health problems across the life span (Benjet, Borges, & Medina-Mora, 2010; Cohen, Janicki-Deverts, & Miller, 2007; Felitti et al., 1998). Yet the mechanisms by which childhood stress exposure undermines long-term health are not fully understood. Epigenetic processes are now thought to be a major mechanism linking early life stress to physical and mental health (Conradt, 2017; Szyf, 2013). The majority of research with children has focused on epigenetic processes related to the hypothalamus–pituitary–adrenal axis stress response system, particularly the glucocorticoid receptor gene, nuclear receptor subfamily 3, group C, member 1 (*NR3C1*). Yet multiple other systems regulate brain function and health. It is well established that serotonin signaling pathways play a key role in brain development, stress reactivity, and mental health (Booij, Tremnlay, Szyf, & Benkelfat, 2015; Lin, Lee, & Yang, 2014), suggesting that epigenetic processes within the serotonin system may also underlie the association of childhood stress exposure and mental health.

Serotonin is a neurotransmitter that acts in the gastrointestinal system, blood, and throughout the brain to influence behavior and physiology. The serendipitous discovery that many of our first psychoactive drugs acted on serotonergic targets has made serotonin a focus of research in psychiatric disorders. Serotonin-producing neurons in the raphe nucleus of the midbrain project to several key regions involved in emotion, behavior, and cognition. From there, released serotonin can bind to over a dozen unique serotonin receptors, each with their own distribution and function. Serotonin receptor 2A (5-HTR2A) has been implicated in multiple psychiatric disorders. Activation of HTR2A receptors in the periaqueductal gray region inhibits paniclike responses, while their activation in the amygdala is related more to generalized anxiety disorder and posttraumatic stress disorder (PTSD) through promotion of inhibitory avoidance and conditioned fear, respectively (Graeff, Viana, & Mora, 1997; Zangrossi et al., 2001). In the prefrontal cortex, activation of 5-HT2A receptors facilitates top-down control of the amygdala, regu-

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Address correspondence and reprint requests to: Stephanie H. Parade, Bradley Research Center, E. P. Bradley Hospital, 1011 Veterans Memorial Parkway, East Providence, RI 02915; E-mail: Stephanie_Parade@Brown.edu.

lating the stress response (Arnsten, 2009; Aznar & Klein, 2013). However, overactivation of cortical 5-HT2A receptors (especially in the context of glutamate receptor hypoactivity) has been linked to psychosis and the effects of hallucinogens (González-Maeso & Sealfon, 2009; González-Maeso et al., 2007).

Multiple postmortem and in vivo binding studies have found changes in 5-HT2A receptor binding and expression in the brains of individuals with various forms of psychopathology. Increases in cortical 5-HT2A receptor binding have been found in suicide victims and in individuals with major depressive disorder (MDD) who did not die by suicide, but results are conflicting (Stockmeier, 2003). Findings also exist for differences in cortical 5-HT2A levels and/or binding affinity in schizophrenia (Hernandez & Sokolov, 2000; Selvaraj, Arnone, Cappai, & Howes, 2014), borderline personality disorder (Soloff et al., 2007), and eating disorders (Frank et al., 2002).

Genetic variation in the 5-HT2A receptor gene (*HTR2A*) may be an important determinant of differences in expression and activity of the 5-HT2A receptor. One of the most widely studied genetic variants of *HTR2A* is the single nucleotide polymorphism (SNP), rs6311 (–1438 A/G). This polymorphism, located in the promoter region of the gene, has been thought to influence gene expression, with initial evidence suggesting that the A allele increases promoter activity and subsequent expression of the 5-HT2A receptor (Parsons, D'Souza, Arranz, Kerwin, & Makoff, 2004; Turecki et al., 1999). The AA genotype has been associated with seasonal affective disorder (Enoch et al., 1999; Lee, Sung, Lim, Paik, & Kim, 2006), bipolar disorder (Bonnier et al., 2002), reduced responses and decreased side effects from antidepressants (Kato & Serretti, 2010), and greater reduction in negative symptoms in response to olanzapine (Ellingrod et al., 2003). For a comprehensive review see Serretti, Drago, and De Ronchi (2007).

In addition to genetic variation, epigenetic modifications play an important role in *HTR2A* regulation. Epigenetic processes include alterations to DNA, which allow for adaptations to the environment but do not change the DNA sequence. Epigenetic modulation of DNA has the potential to alter gene expression, rendering it more or less likely to be expressed, with either positive or negative long-term consequences (Moore, Le, & Fan, 2013; Szyf, 2007). DNA methylation is the most commonly studied epigenetic process, and occurs when a methyl group is added at sites in the DNA where a cytosine nucleotide occurs next to a guanine nucleotide (CpG dinucleotides). Methylation at CpG sites in gene promoter regions may alter transcription factor binding, which may result in transcriptional gene silencing. Consequently, increases in methylation are typically associated with reductions in gene expression, and decreases in methylation are usually associated with increases in gene expression (Egger, Liang, Aparicio, & Jones, 2004; Reik, 2007; Uddin et al., 2011), although the effects of methylation depend on the transcriptional mechanisms at work in the particular region of the gene in question.

Methylation at CpG sites in *HTR2A* can either block or enhance gene transcription by altering binding of transcription factors (Falkenberg, Gurbaxani, Unger, & Rajeevan, 2011). Two CpG sites within the *HTR2A* promoter include those located at –1420 and –1224 base pairs (Falkenberg et al., 2011; Zhu, Chen, & Shih, 1995). While studies investigating the role of *HTR2A* genotype and methylation in psychiatric disorders are limited, hypermethylation has been found in adult schizophrenic and bipolar patients at CpG sites, including –1420, and –1224 (Abdolmaleky et al., 2011; Ghadirivasfi et al., 2011), and it is hypothesized that this might be the mechanism behind findings of decreased *HTR2A* expression in the frontal cortex of patients with schizophrenia (Hurlemann et al., 2007).

Epigenetic changes through methylation are now considered to be a primary mechanism by which the early life environment influences the development of behavioral phenotype. Starting in embryogenesis, serotonin plays an essential role in neural development, with the 5-HT2A receptor promoting neuron proliferation (Azmitia, 2001). Thus, epigenetic changes to *HTR2A* expression via environmental stressors would be expected to influence brain development and, subsequently, behavior. In the neonatal period, methylation of placental *HTR2A* at sites –1420 and –1224 was associated with higher levels of attention in infants (Paquette et al., 2013). Here it is likely that methylation-induced differences in HTR2A receptor expression altered placental physiology, with downstream effects on fetal brain development (Paquette & Marsit, 2014). While this study did not investigate how *HTR2A* expression was influenced in offspring, rodent models have found that both prenatal and postnatal stressors can alter levels of HTR2A receptors in adulthood (Holloway & González-Maeso, 2015; Peters, 1988; Rentesi et al., 2013). As it is unknown how early life experience affects *HTR2A* epigenetics, the present study sought to investigate the relationship between *HTR2A* genotype and methylation as it relates to early life stress and psychiatric symptomatology in a sample of preschoolers with a range of stress exposure.

Method

Data were available from 228 families for the current report. One child from each family was included. Children ranged in age from 3 to 5 years ($M = 50.9$ months, $SD = 9$ months); 121 were female and 107 were male. The sample was racially and ethnically diverse. Eighty-nine children were White, 39 Black, 51 biracial, and 49 other races. One hundred and two children were Hispanic. Most caregivers were biological mothers ($n = 214$). Forty-seven caregivers had less than a high school degree, 88 completed high school, 71 had some postsecondary education, 21 had a bachelor's degree, and 1 did not provide education information. One hundred and twenty-five caregivers in the study were unemployed, and 205 of the families qualified for public assistance. One hundred and nineteen children (52%) had substantiated cases

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of moderate to severe maltreatment within the past 6 months as described below.

Procedure

Families with a maltreated child were identified from the local child welfare agency and an emergency maltreatment assessment service via record review. Families of children with no indicated case of maltreatment were recruited from childcare centers and a pediatric medical clinic during a well-child visit. Based on review of available medical records and parent report, children with a chronic illness, consistent medication use, obesity, or failure-to-thrive were excluded. Those with acute illness or medication use were included no less than 2 weeks following resolution of illness and discontinuation of medication. Families completed a baseline set of assessments at the time of initial study enrollment and a follow-up set of assessments 6 months following enrollment. At each wave of assessment, families completed two home visits and questionnaires between the visits. The current report focuses on the first home visit during the baseline set of assessments, during which caregivers completed interviews on child stress exposure and behavior. A saliva sample for DNA isolation was also collected from the children during this visit.

Measures

Child maltreatment status. All families consented to examination of child welfare records to determine maltreatment status. Trained research staff coded the records using the System for Coding Subtype and Severity of Maltreatment in Child Protective Records (Barnett, Manly, & Cicchetti, 1993). Five maltreatment subtypes and severity scores ranging from 1 (*least severe*) to 5 (*most severe*) were derived. Children with an episode that met the criteria for moderate to severe maltreatment (score of 3–5) within the last 6 months were included in the maltreated group ($n = 119$). Twenty-two children had substantiated cases of physical abuse, 28 sexual abuse, 14 physical neglect/failure to provide, 33 physical neglect/lack of supervision, and 75 emotional maltreatment. The comparison group included children who had never had a substantiated case of maltreatment ($n = 109$).

Contextual stress. Caregivers completed a semistructured interview developed in our laboratory to assess contextual stressors experienced in the child's lifetime. Categories were death of a caregiver, separation from a caregiver, housing instability, inadequate food or clothing, and other stressful events that included witnessing neighborhood violence or parental arrest. Interviews were conducted and scored by trained clinical social workers and PhD-level psychologists. The project coordinator reviewed each interview to ensure compliance with the scoring protocol. Each domain was scored positive if at least one episode occurred, and domains were summed to determine the number of contextual stressor categories the child experienced in his or her lifetime. Possible scores ranged from 0 (*no stressors*) to 5 (*stressors in all five domains*). In the current sample, the number of stressor categories ranged from 0 to 5 ($M = 1.54$, $SD = 1.22$).

Traumatic life events. The Diagnostic Infant and Preschool Assessment (Scheeringa & Haslett, 2010) interview was conducted with caregivers to assess child experiences of traumatic life events. Traumatic events in each domain were dichotomized (no trauma vs. ≥1 trauma), then summed to create a scale for number of types of traumas experienced in the child's lifetime. Physical and sexual abuse were not included because they were assessed as maltreatment (above). Possible scores ranged from 0 (*no traumas*) to 9 (*traumas in all nine domains*). In the current sample, the number of traumatic life events ranged from 0 to 4 ($M = 1.12$, $SD = 1.06$).

PTSD and MDD symptoms. The Diagnostic Infant and Preschool Assessment (Scheeringa & Haslett, 2010) interview was also conducted with caregivers to assess symptoms of PTSD and MDD. Interviews were conducted by trained clinical social workers and PhD-level psychologists, reviewed in a group-supervision format, and scored based upon group consensus. Consistent with the low prevalence of MDD and PTSD diagnoses reported in other studies of children under the age of 6 recruited from low-income mental health clinics (Bufferd, Dougherty, Carlson, & Klein, 2011; Scheeringa & Haslett, 2010), none of the children met full DSM-IV criteria for MDD and only one met criteria for PTSD; therefore, the variables for analysis were the number of symptoms of MDD and of PTSD experienced within the past month. In the current sample, the number of PTSD symptoms ranged from 0 to 10 ($M = 1.44$, $SD = 2.46$) and the number of MDD symptoms ranged from 0 to 3 ($M = 0.41$, $SD = 0.78$).

Behavior problems. Caregivers completed the Child Behavior Checklist for ages 1.5 to 5 (Achenbach & Rescorla, 2000) to assess internalizing and externalizing behavior problems. For each of the 100 behaviors, parents assessed their children on a 3-point scale from 0 (*not true*) to 2 (*very true*). We used T scores for data analysis. The Child Behavior Checklist is a reliable and valid measure with strong test–retest reliability ($r = .90$ and .87 for internalizing and externalizing scales, respectively) as well as discriminant validity between children who were and were not referred for behavioral health services (Achenbach & Rescorla, 2000). In the current sample, the range for internalizing problems was 29 to 79 ($M = 52.84$, $SD = 8.21$) and for externalizing problems was 28 to 78 ($M = 47.50$, $SD = 11.54$).

Methylation of HTR2A. Two CpGs (–1420 and –1224) were studied based on findings of Falkenberg et al. (2011) and Paquette et al. (2013). Saliva samples were obtained using the Oragene DISCOVER kits (OGR-575) for Assisted Collections (DNA Genotek, Kanata, Ontario, Canada), and DNA was isolated following the manufacturer's instructions. A 276 base pair region of the *HTR2A* promoter region (chromo-

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some 7: 28415063-28414801) was amplified from bisulfite modified DNA using the pyromark PCR kit (Qiagen, 203443), and DNA methylation assessed through pyrosequencing following a serial pyrosequencing protocol using a PyroMark MD system (Qiagen) and two sequencing primers as described in Paquette et al. (2013). Each pyrosequencing assay contained a bisulfite conversion control to assess conversion efficiency. All samples examined demonstrated >95% conversion.

HTR2A genotyping. Genotyping of the *HTR2A* (rs6311) SNP, rs6311 (–1438 A/G), was determined visual inspection of the pyrogram resulting from the first pyrosequencing assay. Thirty-five children were homozygotes for the A allele, 121 children were AG heterozygotes, and 72 children were homozygotes for the G allele.

Modeling ancestry differences using principal component analysis. Allele frequency differences due to systematic ancestry differences could cause spurious associations. We used principal components analysis to model ancestry differences in the current study using genome-wide SNP markers from saliva DNA genotyped using the Illumina Infinium PsychArray-24 beadchip (over 588,000 autosomal SNPs). Genotypes were cleaned using standard quality-control procedures. We conducted the linkage disequilibrium-based pruning first, and followed by principal components analysis using PLINK (Purcell et al., 2007). Linkage disequilibrium-based pruning reduces correlation among SNPs such that the principal components (PCs) of the genetic variation in the sample would not be overweighted by the contribution of correlated SNPs. The first two PCs obtained using PLINK were used for controlling the potential population stratification (Price et al., 2006).

Statistical analysis

Mean differences in demographic characteristics, stress exposure, and child behavior based on genotype were examined using *t* tests and chi-square. Simple correlations between demographic characteristics and methylation were conducted to determine inclusion of covariates. General linear modeling was used for hypothesis testing. Two PCs to adjust for genetic ancestry were included in the general linear models as a priori covariates. Child age was associated with genotype and methylation as described below, and therefore was also included in the models as a covariate. *HTR2A* genotype was associated with methylation as described below, and was included as a covariate in models testing main effects of stress exposures and child symptoms and behavior on methylation. Outliers, defined as values more than 3 *SD* from the mean, were Winsorized by setting them to the next highest value within 3 *SD*. Missing data were imputed using SPSS 22. Less than 3% of data was missing overall. Little's missing completely at random test (Little, 1988) demonstrated that the data were missing completely at random. Ten imputed data sets were derived from the original data set, analyses were run on each individual data set, and parameter estimates from all 10 data sets were pooled to derive the final results. The results derived using multiple imputation were consistent with those derived from the original data set with missing values, with the exception that the effect of MDD symptoms on methylation at –1420 was at trend level ($p < .10$) using the original data set and reached significance using the imputed data set.

Results

Sample characteristics

The minor allele frequency of the *HTR2A* allelic variant in the sample was .42 and the distribution conformed to the Hardy–

Table 1. *Descriptive statistics and mean differences by serotonin receptor 2A (HTR2A) genotype*

	AA ($n = 35$)	AG ($n = 121$)	GG ($n = 72$)	*p*
Sex, *N* (%) female	20 (57.1)	63 (52.0)	38 (52.7)	.87
Age, *M* (*SD*)	4.5 (0.7)[a]	4.1 (0.7)[a]	4.3 (0.8)	.02
PC for genetic ancestry 1, *M* (*SD*)	−0.02 (0.1)	0.00 (0.1)	0.01 (0.1)	.11
PC for genetic ancestry 2, *M* (*SD*)	0.02 (0.1)	−0.01 (0.1)	0.00 (0.1)	.16
Maltreatment status, *N* (%)	20 (57.1)	65 (53.7)	34 (47.2)	.56
Number of stressors, *M* (*SD*)	1.7 (1.2)	1.5 (1.2)	1.6 (1.2)	.60
Traumatic life events, *M* (*SD*)	1.06 (1.0)	1.17 (1.1)	1.07 (1.1)	.74
PTSD symptoms, *M* (*SD*)	1.8 (2.8)	1.3 (2.3)	1.5 (2.6)	.57
MDD symptoms, *M* (*SD*)	0.6 (1.1)	0.4 (0.7)	0.4 (0.7)	.31
Internalizing behavior, *M* (*SD*)	52.2 (7.3)	53.3 (8.5)	52.4 (8.2)	.71
Externalizing behavior, *M* (*SD*)	48.8 (11.5)	47.1 (11.4)	47.6 (11.8)	.74

Note: The *p* values indicate the *t*-test or chi-square significance level. PC, principal component; PTSD, posttraumatic stress disorder; MDD, major depressive disorder.
[a]Values are significantly different at $p < .01$.

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Weinberg equilibrium ($\chi^2 = 1.85$, $p = .17$). Table 1 shows sample characteristics in relation to *HTR2A* genotype. A homozygotes were significantly older than heterozygotes. Child sex, the PCs to adjust for genetic ancestry, stress exposure, and behavior did not differ according to genotype. Child age was not associated with methylation at –1420, but was negatively associated with methylation at –1224 ($r = –.14$, $p = .034$). Child sex and the PCs to adjust for genetic ancestry were not associated with methylation at –1420 or –1224.

HTR2A genotype effects on methylation

HTR2A genotype was associated with methylation at –1420, $F (2, 225) = 8.33$, $p < .001$, and –1224, $F (2, 225) = 43.77$, $p < .001$. As displayed in Figure 1, A homozygotes had lower methylation at –1420 than heterozygotes and G homozygotes. A homozygotes also had lower methylation at –1224 than heterozygotes and G homozygotes, and heterozygotes had lower methylation at –1224 than G homozygotes.

Stress exposure and methylation

Main effects of child maltreatment status, traumatic life events, and contextual stress on methylation at –1420 and –1224 were not significant. The interaction of contextual stress and genotype was a significant predictor of methylation at –1420, $F (2, 219) = 3.52$, $p = .031$. To understand the interaction effect, we examined partial correlations of contextual stress and –1420 methylation within each genotype, controlling for age and the PCs to adjust for genetic ancestry. As illustrated in Figure 2, contextual stress was positively associated with methylation among A homozygotes ($r = .21$), negatively associated with methylation among G homozygotes ($r = –.28$), and not associated with methylation among heterozygotes ($r = .02$). In contrast, the interaction of child maltreatment status and genotype was not significant, nor was the interaction of traumatic life events and genotype.

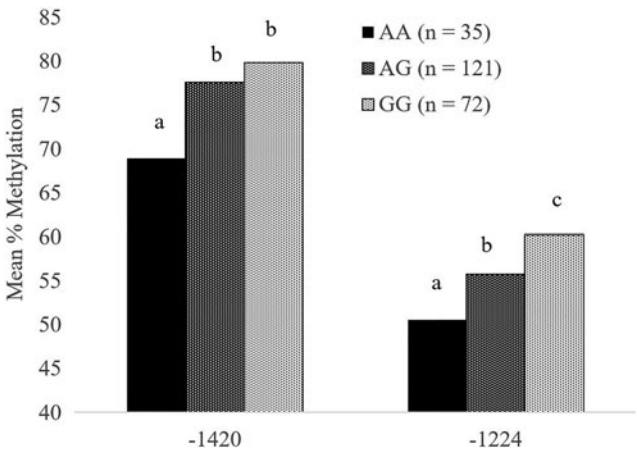

Figure 1. Mean differences in methylation by serotonin receptor 2A (*HTR2A*) genotype. Different letters indicate significant differences within the CpG site based on genotype at $p < .01$.

Child symptoms and methylation

Main effects of PTSD symptoms, $F (1, 221) = 5.35$, $p = .022$, and MDD symptoms, $F (1, 221) = 5.16$, $p = .024$, on methylation at –1420 were significant. Likewise, main effects of PTSD symptoms, $F (1, 221) = 11.06$, $p = .001$, and MDD symptoms, $F (1, 221) = 6.30$, $p = .013$, on methylation at −1224 were significant. Examination of partial correlations controlling for age, the PCs to adjust for genetic ancestry, and *HTR2A* genotype indicated that PTSD and MDD symptoms were negatively associated with methylation at −1420 ($r = −.15$, $p = .022$ for both PTSD and MDD symptoms), but positively associated with methylation at –1224 ($r = .22$, $p = .001$ for PTSD symptoms and $r = .17$, $p = .014$ for MDD symptoms). In contrast, interactions of PTSD and MDD symptoms with genotype were not significant predictors of methylation at –1420 or –1224.

Child behavior problems and methylation

Internalizing and externalizing behavior problems were not associated with methylation at –1420 or –1224. Likewise, interactions of *HTR2A* genotype and internalizing and externalizing behavior problems were not significant predictors of methylation at –1420 or –1224.

Discussion

The current study examined methylation of *HTR2A* with early life stress and psychiatric symptoms in a sample of preschoolers with early adversity. We demonstrated that *HTR2A* genotype is associated with *HTR2A* methylation at CpGs –1420 and –1224, such that A homozygotes had lower methylation than children with the G allele. *HTR2A* genotype also moderated associations of contextual stress exposure and *HTR2A* methylation at –1420. Contextual stress was positively associated with –1420 methylation among A homozygotes, but negatively associated with –1420 methylation among G homozygotes. PTSD and MDD symptoms were negatively associated with methylation at –1420, but positively associated with methylation at –1224. Collectively, these results suggest that *HTR2A* is sensitive to stress exposure and psychopathology, and that environmental factors work in conjunction with DNA variation to affect this epigenetic process.

In the present study, we found decreased methylation at both the –1420 and –1224 CpG sites in those with the AA genotype. Few studies have examined this issue, but initial evidence has suggested that the A allele results in increased *HTR2A* expression (Parsons et al., 2004; Turecki et al., 1999; but see Bray, Buckland, Hall, Owen, & O'Donovan, 2004; Smith et al., 2013, for alternate evidence). Given that methylation typically functions to silence gene expression (Egger et al., 2004), the decreased methylation in AA individuals found in the present study might facilitate increased gene expression associated with this allele. As the present study measured methylation of two CpG sites in *HTR2A* in

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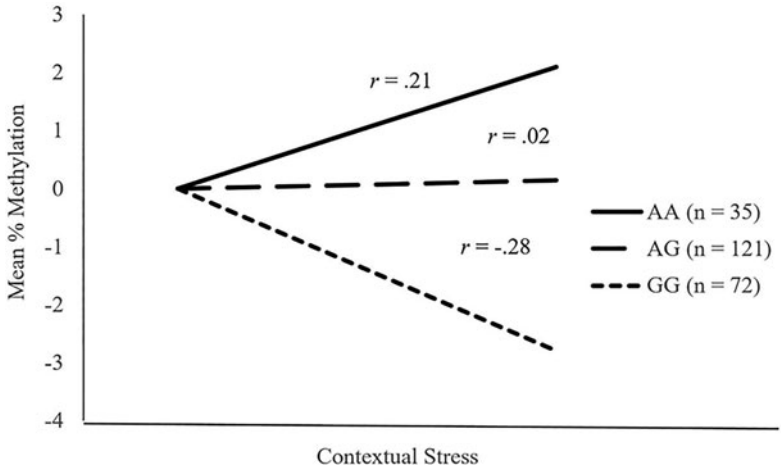

Figure 2. Serotonin receptor 2A (*HTR2A*) genotype moderates the association of contextual stress and *HTR2A* –1420 methylation.

saliva DNA and did not evaluate gene expression, it is unknown how such differences in methylation might affect receptor expression within the brain.

While the data on the relationship between *HTR2A* methylation and receptor expression is limited, Falkenberg et al. (2011) created a model of *HTR2A* regulation based on a combination of computational, animal, and human evidence. In this model, decreased methylation in the AA genotype at –1420 and –1224 allows for binding of the glucocorticoid receptor and transcription factor SP1, respectively. These transcription factors have opposing actions on gene expression, with the glucocorticoid receptor acting as a repressor and SP1 as a promoter. This model suggests that our findings of decreased methylation at –1420 and –1224 in AA individuals could have mixed rather than unilateral effects on gene expression.

Contextual stress was associated with increases or decreases in methylation at –1420 dependent on genotype. In AA individuals, contextual stress was associated with increased methylation while the opposite was true in GG individuals. Based on the model of Falkenberg et al. (2011), by increasing methylation of the AA genotype (and blocking the repressive glucocorticoid response element), one would expect increased expression, while the decreased methylation in the GG genotype reduces expression. That contextual stress resulted in a differing propensity for methylation based on genotype suggests a mechanism for potential Gene × Environment interaction. While the details and consequences of such an interaction would require further study, it is known that other serotonin genes demonstrate different methylation based on stress exposure and genotype. For example, early life and recent stress has been correlated with higher levels of methylation in individuals with the SS version of the serotonin transporter gene, solute carrier family C6, member 4 (*SLC6A4*; Duman & Canli, 2015) with this genotype being linked to higher stress sensitivity (Karg, Burmeister, Shedden, & Sen, 2011).

Animal models support a role for stress in altering *HTR2A* expression, although results are conflicting. Prenatal stress has been found to result in both increased and decreased *htr2a* receptor expression in adult offspring (Holloway & González-Maeso, 2015; Peters, 1988; Rentesi et al., 2013). Postnatal maternal deprivation stress has been shown to result in decreased *HTR2A* expression in the frontal cortex of rats (Rentesi et al., 2013), while repeated exposure to inescapable shock in adulthood increased *HTR2A* expression (Dwivedi, Mondal, Payappagoudar, & Rizavi, 2005). As these studies deal with stress exposure occurring during discrete periods of development, they have limited applicability to the contextual stress measurement in the present study. That child maltreatment and traumatic life events were not associated with *HTR2A* methylation, but contextual stress exposure was associated with methylation in conjunction with genotype, suggests that developmental timing and other stressor characteristics are possible determinants of *HTR2A* methylation and gene expression. Further research is needed to understand these processes.

Although overall symptom levels of MDD and PTSD were low in our sample, we did find significant associations with *HTR2A* methylation. To our knowledge, we are the first to show a relationship with PTSD and MDD symptoms and methylation of *HTR2A* –1420 and –1224 CpG sites. Specifically, symptom levels were positively correlated with methylation at the –1420 site and negatively correlated with symptoms at the –1224 site. This pattern of methylation would enhance binding of the promoter transcription factor Sp1 (Zhu et al., 1995) while decreasing that of the repressive glucocorticoid receptor (Falkenberg et al., 2011) and therefore might be expected to increase overall expression. This is in line with a recent study demonstrating that expression of *HTR2A* mRNA in peripheral blood cells was associated with greater depression severity (Amidfar et al., 2017). The current study did not find a relationship between genotype and MDD or PTSD symptoms. While there is a lack of data on PTSD, recent meta-analyses have found both no relation-

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ship between –1438 A/G polymorphism and MDD (Jin, Xu, Yuan, Wang, & Cheng, 2013) and increased risk with AA genotype (Zhao et al., 2014). These conflicting results might be reconciled with additional studies that take into account methylation and contextual stressors, as our results suggest these play a role in genotype-dependent regulation of the 5-HT2A receptor.

The current study is the first to examine associations of early life stress and psychopathology with *HTR2A* methylation. Nonetheless, there are limitations that warrant future research in this domain. First, our comparison group of children with no maltreatment history was exposed to a range of other contextual stressors associated with poverty that may have reduced the strength of associations among maltreatment and methylation. Future work should draw upon a comparison group of children with no maltreatment history or other contextual stressors to determine if the nonsignificant associations among maltreatment and *HTR2A* methylation are unique to our sample with a range of other stress exposures. Likewise, although child protection records were reviewed for all children in the study, it is possible that children within the comparison group had undocumented maltreatment, further reducing the strength of associations. Second, the current study focused on contextual risk factors and psychopathology as they relate to methylation and is an important first step in understanding environmental effects on *HTR2A* methylation. Examination of protective factors including parenting sensitivity and engagement in therapeutic interventions that potentially enhance resilience among children exposed to early adversity is an important next step. Third, and as discussed above, data on gene expression is not currently available from the sample and would be useful in understanding the mechanisms underlying our results. Despite these limitations, the current study provides novel data linking stress exposure and psychiatric symptoms to *HTR2A* methylation in a sample of children with early adversity. Results support the perspective that DNA methylation is a mechanism by which early life stress impacts child mental health, and implicates serotonin signaling pathways as a relevant epigenetic process.

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