Additive serotonergic genetic sensitivity and cortisol reactivity to lab-based social evaluative stress: Influence of severity across two samples

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Abstract:

Prior work demonstrates that an additive serotonergic multilocus genetic profile score (MGPS) predicts amplified risk for depression following significant life stress, and that it interacts with elevations in the cortisol awakening response to predict depression. The serotonin system and HPA-axis have bidirectional influence, but whether this MGPS predicts acute cortisol reactivity, which might then serve as a mechanism for depression, is unknown. Our prior work suggests that depression risk factors predict blunted cortisol reactivity to explicit negative evaluative lab-based stress. Thus, we hypothesized that a 4-variant serotonergic MGPS (three SNPs from the original 5-variant version plus 5HTTLPR) would predict blunted cortisol reactivity to explicit negative evaluative stress versus a control. In Sample 1, growth curve modeling showed that the MGPS predicted heightened cortisol reactivity ($p = 0.0001$) in an explicitly negative evaluative Trier Social Stress Test variant (TSST) versus a control condition among non-depressed emerging adults ($N = 152$; 57% female). In Sample 2, 125 males completed the Socially Evaluative Cold Pressor Test (SECPT), an ambiguously negative evaluative manipulation; findings displayed a similar pattern but did not reach statistical significance (ps.075–.091). A participant-level meta-analysis of the two samples demonstrated a significant effect of negative evaluation severity, such that the MGPS effect size on reactivity increased linearly from control to SECPT to an explicitly negative evaluative TSST. Findings indicate that this MGPS contributes to sensitivity to social threat and that cortisol dysregulation in the context of social stress may be one mechanism by which this MGPS contributes to depression.

Keywords: serotonin | cortisol reactivity | multilocus genetic profile score | emerging adults | lab-based stress | negative evaluative

Article:

1. Introduction

Additive Serotonergic Genetic Sensitivity and Cortisol Reactivity to Lab-Based Social Evaluative Stress: Influence of Severity Across Two Samples Although single candidate gene research has faced substantial criticism (Border et al., 2019, Culverhouse et al., 2018), newer efforts have examined additive genetic scores that better conform to polygenic theoretical assumptions for psychopathology (Bogdan et al., 2018). An additive multilocus genetic profile score (MGPS) using five variants specific to the serotonin system (other than the more frequently studied transporter polymorphism, 5HTTLPR) has been shown in three independent samples to predict depression as a function of recent stress exposure (Starr et al., 2019, Vrshek-Schallhorn et al., 2015b), and to be specifically sensitive to interpersonal stress as opposed to non-interpersonal stress (Starr et al., 2019). Further, in each of these three samples, there was evidence that this MGPS conferred sensitivity to the environment "for better or for worse," consistent with the differential susceptibility hypothesis (Belsky and Pluess, 2009). That is, the MGPS not only conferred elevated risk of depression under adverse conditions, but also reduced risk under better conditions, leading us to frame these variants as hypothesized "sensitivity" alleles. Although this work has exclusively examined depression as an outcome, these sensitivity alleles are highly likely to act via intermediate outcomes.

One such intermediate outcome is stress reactivity in the hypothalamic-pituitary-adrenal (HPA) axis, based on evidence of substantial physiological connectivity with the serotonin system. For example, cortisol inhibits serotonin (Leitch et al., 2003, Maes et al., 1990, Rubin, 1967, Tafet et al., 2001), and serotonin can both stimulate and inhibit (under varying conditions) cortisol release via the 5HT-1A, − 2A, and − 2 C receptors in the hypothalamus, adrenal glands, and pituitary, among others (Lowry, 2002). Recent work showed that both the original 5-variant genetic score, a 6-variant version incorporating 5HTTLPR, and 5HTTLPR alone significantly interact with the magnitude of cortisol's rise upon natural waking, the cortisol awakening response, to prospectively predict depressive episode onset (Vrshek-Schallhorn et al., 2015a), in a manner consistent with differential susceptibility. Further, meta-analytic evidence indicates that 5HTTLPR predicted increased cortisol reactivity in response to lab-based stress (Miller et al., 2013). Extending this finding to the novel serotonergic MGPS would further implicate HPA-axis dysregulation in the MGPS's mechanism of stress-induced depression, but this is untested.

Regarding the direction of effect of the MGPS on cortisol reactivity, in the approximately fifteen years of research on individual differences predicting cortisol reactivity to controlled stress, most researchers initially hypothesized that depression risk factors would predict heightened reactivity. This prediction, however, has not unilaterally born out in the data: Such risk factors have produced both heightened and blunted patterns of reactivity in response to lab-based stress (for a review, see Phillips et al., 2013). Some of us have proposed in the Cortisol Reactivity Threshold Model that the severity of the lab-based manipulation, indexed by the level of negative evaluation, may account for the disparate findings in direction of the risk factor-reactivity relationship; we demonstrated this with the internalizing risk factor trait rumination (Vrshek-Schallhorn et al., 2018). In this view, internalizing risk factors would be associated with greater severity perception of all manipulations, and perceptions of modest severity would associate with increased motivation while perceptions of excessive evaluation would associate with demotivation.

Conceptualizing cortisol as resource-mobilizing, this model predicts that moderate stress produces a positive correlation between the risk factor and cortisol reactivity (suggesting an

excessive response by those at-risk), whereas robust stress produces a negative correlation (indicating a "giving up" physiological withdrawal by those at-risk, while those less at-risk "rise to the occasion"). Non-stressful circumstances would produce no association. Despite evidence that one serotonin genetic variant, 5HTTLPR, produced greater cortisol reactivity in a meta-analysis of primarily moderate threat manipulations (Miller et al., 2013), and other evidence that supports that both augmented and blunted cortisol reactivity represent HPA-axis dysregulation (Foley and Kirschbaum, 2010), this theoretical perspective suggests the serotonergic MGPS would predict a blunted response to robust negative evaluative stress.

Here, in a first sample (Sample 1), four genetic variants from the novel MGPS previously identified for their association with depression (rs6314 in HTR2A, rs6295 in HTR1A, rs4570625 in TPH2, and 5HTTLPR) were available from participants in two experimental conditions, a negative evaluative version of the Trier Social Stress Test and a non-stressful control, as reported in prior work using three severity levels of the TSST (Vrshek-Schallhorn et al., 2018). Of these, the influence of rs6295 on lab-based cortisol reactivity has been reported previously, indicating that the G-allele predicts blunted cortisol responding in older adults (Armbruster et al., 2011) and the 5HTTLPR S-alleles have been implicated in heightened cortisol responding to stress in a meta-analysis (Miller et al., 2013). In an independent second sample (Sample 2) identified to replicate results that contradicted initial hypotheses for Sample 1, all participants completed the Socially Evaluative Cold Pressor Test, an ambiguously evaluative stress induction, with an experimenter protocol similar to the Intermediate level in prior work using three severity levels of the TSST (Vrshek-Schallhorn et al., 2018). In a combined participant-level meta-analysis of both samples, we then capitalize on differences in the severity of stress inductions across the two samples' manipulations to test a hypothesis that negative evaluation level would predict greater MGPS effect on cortisol reactivity.

Primarily functional markers were originally chosen for the MGPS based on prior reports of association with internalizing psychopathology (Vrshek-Schallhorn et al., 2015b); a brief review of the four variants in the present reports is merited. First, a functional C to G promoter polymorphism rs6295 for 5-HT1A, a negative autoreceptor that downregulates serotonergic activity, impacts both receptor density and binding potential (Lemonde et al., 2003). The G allele (GG or G-Carrier) is linked to depression risk (Anttila et al., 2007, Lemonde et al., 2003) and to comorbid generalized anxiety with depression (Molina et al., 2011), but also impaired antidepressant response (e.g., Hong et al., 2006; Lemonde et al., 2004). Second, a functional C to T polymorphism rs6314 in the gene encoding the 5-HT2A receptor does not appear to impact binding affinity; however, the rarer T-allele carriers display blunted calcium signaling compared to the more common C-allele homozygotes (Ozaki et al., 1997). Although the T-allele has been implicated in impaired memory recall (de Quervain et al., 2003, Wagner et al., 2008), it was also implicated in a protective effect in studies of suicidal ideation and attempt (although was not significant in the broadest analysis) (Li et al., 2006), providing an early suggestion that the C-allele is riskier for internalizing psychopathology.

Third, although not functional (Scheuch et al., 2007), the TPH2 (tryptophan hydroxylase-2, the primary catalyzing enzyme in the brain for the rate-limiting step in serotonin production) SNP rs4570625 (specifically, the G-allele) was the only TPH2 SNP of ten to achieve a stringent level of significance in a meta-analysis predicting greater risk for major depression (Gao et al., 2012). This was further supported by later work indicating that individuals homozygous for T/T experience less depression, anxiety, and aggression than their T/G and G/G counterparts (Laas et al., 2017), as well as a later meta-analysis (Liu et al., 2021). Fourth and finally, although

controversial (Culverhouse et al., 2018, Karg et al., 2011), 5HTTLPR is a functional insertion/deletion promoter region polymorphism near the serotonin transporter gene, SLC6A4, for which the short allele (S) is less transcriptionally efficient (potentially resulting in reduced reuptake and greater serotonergic tone in the synaptic cleft) than the long (L) allele (Heils et al., 1996). In meta-analyses, the S-carrier genotype has been shown to predict greater cortisol reactivity to lab-induced stress (Miller et al., 2013), diminished response to antidepressants (Serretti et al., 2007), and is implicated in differential susceptibility among children and adolescents (Van IJzendoorn et al., 2012). Further, the S-allele is implicated in neural activation under induced stress in several brain regions involved in the etiology of depression (Sun et al., 2020).

Thus, for the first time, the present study examined how a serotonergic MGPS comprising four genetic variants located in or adjacent to HTR2A, HTR1A, and TPH2, and 5HTT predicts cortisol reactivity in the context of socially evaluative stress and as a function of level of negative evaluation, to extend work showing that a serotonergic MGPS is sensitive to the environment both for better or for worse in predicting depression (Starr et al., 2019, Vrshek-Schallhorn et al., 2015b).

2. Sample 1

2.1. Methods

2.1.1. Participants

Undergraduates (N = 152; 57% female, ages 18–29) at a midsized Southeastern U.S. public university were recruited to participate in a study on genetics and lab-based stress. Eligible participants were between the ages of 18–30, denied current use of hormonal birth control, nicotine, steroidal or psychotropic medication, and denied chronic health conditions. Additional exclusion criteria unrelated to the current aims were high blood pressure, colorblindness, and head trauma history. Finally, individuals meeting criteria for current depression were diverted from the stress condition to a control condition, and are excluded from the present analyses due to non-randomization and that current depression alters cortisol response (Burke et al., 2005).

Participants identified as Black/African American (n = 68, 44.7%), White (n = 58, 38.2%), Hispanic/Latin(a/o) (n = 8, 5.3%), Asian (n = 6, 3.9%), biracial (n = 4, 2.6%), and other races and ethnicities (n = 8, 5.3%). The challenge condition comprised n = 77 (57% female), while the control comprised n = 75 (57% female).

Participants were enrolled into a single protocol in two phases (first n = 59, second n = 93) that used different cognitive tests following stress, not reported here. The first phase also included a third smaller intermediate condition who did not provide DNA. Phase 1 was used in our prior report regarding cortisol reactivity and trait rumination (Vrshek-Schallhorn et al., 2018).

2.2. Materials and procedures

All participants provided informed, signed consent and debriefing; all procedures were approved by the IRB of the University of North Carolina at Greensboro (UNCG).

2.3. DNA collection, genotyping, and sensitivity score construction

Participants provided saliva samples for DNA extraction and genotyping in sterile DNAse and RNAse-free cryogenic vials. After collection, saliva samples were stored at − 80 °C. DNA was extracted using Oragene extraction kits (DNA Genotek, Ontario, Canada) and genotyped at the UNCG Molecular Core Lab, with the exception of 5HTTLPR, which was genotyped at the University of Wisconsin-Madison Biotechnology Center following previously reported methods (Wendland et al., 2006). Following prior work using this serotonergic MGPS, rs25531 was not used to recode 5HTTLPR (Hu et al., 2005) due to concerns about the replicability of this finding (Martin et al., 2007, Philibert et al., 2008).

Six serotonin variants were genotyped in Sample 1 for potential inclusion, and three did not conform to Hardy Weinberg Equilibrium (HWE; $p < .05$, rs6318 checked only in females due to X-linkage, 5HTTLPR, and rs1117899). Further inspection suggested that TPH2 rs1117899 genotypes were inaccurate, while heterogeneous ancestry may have accounted for HWE departures in 5HTTLPR and rs6318 (the latter unavailable in Sample 2). The remaining three, rs6314, rs4570625, and rs6295, conformed to HWE ($\chi 2s \leq 1.699$, $ps \geq .192$). For consistency across the two samples, for the primary analyses, an MGPS was constructed from rs6314, rs4570625, rs6295, and 5HTTLPR, and secondary sensitivity analyses in Sample 1 probed whether the pattern of findings persisted when dropping 5HTTLPR or adding in HTR2C rs6318.1 The four variants available were used to compute the MGPS as described in a prior report (Vrshek-Schallhorn et al., 2015b), where all were coded for number of sensitivity alleles, 0–2 (designated as C for rs6314, G for rs4570625, and G for rs6295), with the exception of 5HTTLPR which was coded as S-carriers (0 = LL, 1 = SL or SS), following prior MGPS coding (Vrshek-Schallhorn et al., 2019b). Sensitivity alleles were summed (observed range 1–7, M = 4.42, SD = 1.14). We permitted 1 missing genotype (n = 29 participants), prorating MGPS scores using available data; one person was missing 2 of 4 genotypes and was excluded from MGPS analyses but permitted in individual SNP analyses. Analyses were conducted using all available data, and thus, individual analyses varied slightly in N, with the primary analyses comprising 151 participants.

2.4. Stress manipulations and cortisol

All participants were asked to abstain from eating for 30 min prior to the study, and to abstain from exercising, caffeine, tobacco, and alcohol/drug use for one hour prior to arriving. Participants were pseudorandomized to either an explicit negative evaluative adaptation (Way and Taylor, 2010) of the Trier Social Stress Test (TSST; Kirschbaum et al., 1993) or a non-evaluative control (Way and Taylor, 2010). In both, participants were told they would be video-recorded (but were actually not) and instructed to face the camera. They had 5 min to prepare a 5-minute speech. Following the speech, participants completed a 5-minute serial subtraction task counting backwards from 2017 by 13′s. Upon errors, they were instructed to start back from 2017 again. Conditions differed in several ways. The negative evaluative TSST included judges who displayed scripted bored or dissatisfied non-verbal behaviors and a negative evaluative tone, while the control had a polite, non-evaluative experimenter and no judges. Further, the negative evaluative condition utilized a more self-evaluative speech topic compared to the control. Full details are reported in a prior sample using the same two conditions (Avery and Vrshek-Schallhorn, 2016).

All participants provided saliva for cortisol at 4 time points: immediately prior to the TSST (baseline), + 20 min (following the TSST), + 45 min, and + 65 min (following debriefing and rest).

A sample collected at + 5 min was not included as this would be too early to detect changes in cortisol. Immediately after the TSST, all participants completed manipulation checks assessing perceived overall, positive, and negative evaluation. To avoid confounds from high morning cortisol levels, study sessions began between 1 pm and 4 pm.

2.5. Analytic plan

Limited missing data in psychometric data, covariates, and cortisol were addressed with a multiple imputation procedure (Yuan and Bentler, 2000). Manipulation checks were examined using one-way ANOVAs in SPSS 26 (IBM Corp, USA). Remaining models utilized multilevel growth curve modeling in SAS 9.4 (Cary, NC, USA), which accounts for the nested nature of repeated cortisol measurements within-person over time. This approach permits examination of growth curves that separately model increases/decreases (Linear Time), and the parabolic rise and fall (Quadratic Time) of the dependent variable (Hedeker and Gibbons, 2006), the latter reflecting cortisol reactivity (e.g., Avery and Vrshek-Schallhorn, 2016). The primary hypothesis was tested with a three-way interaction of MGPS × Stress × Quadratic Time. Time was modeled with orthonormalized contrasts to prevent correlation of linear and quadratic effects (Hedeker and Gibbons, 2006). Based on preliminary examination, models included intercept and linear time slope random effects. Dimensional independent variables were grand mean centered.

The primary model is reported without covariates; in the event of significant findings, a priori covariates for a follow-up model were gender (female = 0, male = 1), socioeconomic status (SES) using Hollingshead's measure (1975), and racial/ethnic self-identification as a person of color (1 = yes, 0 = no). All covariates in this follow-up test were covaried as main effects and as interactions with all terms necessary to mathematically partial their influence from the MGPS x Stress x Quadratic Time primary effect, following an emerging convention in gene-by-environment interaction research (Keller, 2014). Additional follow-up models examined whether a significant effect held in several subgroups: the two study phases, and Black/African American- and White-identifying participants, the largest two racial/ethnic subsamples.

Planned post-hoc analyses in the event of significant findings were to: (a) use simple effects to test the MGPS x Quadratic Time interaction in each condition, and (b) re-run the primary model substituting each individual single nucleotide polymorphism (SNP) for the MGPS variable. The latter examined whether all effects contributed to the additive effect by producing effect sizes in the same direction, and whether any produced descriptively larger effects.

2.6. Results

2.6.1. Preliminary analyses

Table 1 provides group descriptives. The stress conditions did not vary in gender or racial/ethnic-identification, all $\chi 2 \leq 1.46$, $p \geq .227$, nor in age or baseline cortisol levels, all $F(1150) \leq 1.19$, $p \geq .278$. However, the stress condition had somewhat lower SES than controls, $F(1150) = 3.87$, $p = .051$, and somewhat higher MGPS than controls $F(1149) = 2.89$, $p = .091$ (see Table 1). To address the former, follow-up models covaried SES; regarding the latter, follow-up analyses examined the effect of MGPS within each condition.

Table 1. Sample 1 Characteristics by Stress Condition.

	Control	Negative evaluative TSST
	Mean (Standard deviation)	
Age	19.44 (1.75)	19.42 (2.09)
SES Index	45.81 (12.75)	41.65 (13.31)
MGPS (range 1-7)	4.26 (1.24)	4.58 (1.02)
LN Baseline Cortisol	1.57 (0.51)	1.48 (0.48)

Note: SES Index = Hollingshead Socioeconomic Status Index; LN = Natural log transformed; MGPS = multilocus genetic profile score.

Manipulation checks showed that the TSST functioned as expected: Cortisol reactivity was significantly greater in the negative evaluative condition versus the control condition (Stress x Quadratic Time, $t = -6.15$, $p < .0001$). Further, compared to controls, the negative evaluative group reported feeling more evaluated generally ($F(1150) = 18.257$, $p < .001$), and among those who felt evaluated at all, more negatively evaluated ($F(1134) = 57.371$, $p < .001$) and less positively evaluated ($F(1134) = 39.206$, $p < .001$).

2.6.2. Primary results

The primary growth curve analysis indicated that the MGPS significantly moderated the relationship between stress condition and cortisol reactivity, as indicated by the MGPS × Stress × Quadratic Time interaction, $b = -0.1628$, $SE(b) = 0.04234$, $t(298) = -3.85$, $p = 0.0001$ (Table 2a). Fig. 1 indicated that MGPS was associated with greater cortisol reactivity in the negative evaluative TSST, counter to hypotheses.

This three-way interaction was reproducible in the first study phase, $b = -0.205$, $SE(b) = 0.071$, $t(114) = -2.87$, $p = 0.0048$, as well as in the second study phase, $b = -0.139$, $SE(b) = 0.054$, $t(180) = -2.57$, $p = 0.0111$ (Supplement Table S1). With covariates added for gender, SES, and race/ethnicity, including both their main effects and the interactions (e.g., Covariate x Stress) necessary to partial their effects from the three-way interaction, the MGPS × Stress × Quadratic Time remained significant, $b = -0.188$, $SE(b) = 0.044$, $t(291) = -4.30$, $p < .0001$ (Supplemental Table S2). Further, although self-reported race/ethnicity is not a strong proxy for genetic ancestry, the primary interaction effect persisted (separately) with similar effect sizes in the two largest subgroups, Black/African American-identifying participants, $b = -0.141$, $SE(b) = 0.067$, $t(130) = -2.09$, $p = 0.0382$, and White-identifying participants, $b = -0.2429$, $SE(b) = 0.06838$, $t(112) = -3.55$, $p = 0.0006$ (Supplement Table S3). This suggests population stratification did not spuriously produce results.

2.6.3. Effect decomposition

Simple effect post-hoc tests indicated that the MGPS x Quadratic Time interaction was significant among the negative evaluative group, $b = -0.122$, $SE(b) = 0.0325$, $t(298) = -3.74$, $p = 0.0002$, but not among controls, $b = 0.041$, $SE(b) = 0.027$, $t(298) = 1.52$, $p = 0.1295$.

To further decompose results, we repeated the primary model with each constituent SNP alone (coded as 0–2 "sensitivity" alleles) in separate models. All four SNPs produced effect sizes for their respective SNP x Stress x Quadratic Time interaction in the same direction, suggesting each contributed to the overall pattern of results of the additive MGPS variable. Two SNPs

Table 2.

a. Sample 1. MGPS primary model.

	b	SE (b)	DF	t-value	p-value
Intercept	1.4021	0.05172	298	27.11	< .0001
Linear Time	-0.2900	0.05211	147	-5.57	< .0001
Quadratic Time	-0.0243	0.03395	298	-0.72	0.4734
Stress Condition	0.3072	0.07279	298	4.22	< .0001
MGPS	0.0333	0.03186	298	1.05	0.2967
Stress x Linear Time	0.3494	0.07313	298	4.78	< .0001
Stress x Quadratic Time	-0.2795	0.04764	298	-5.87	< .0001
MGPS x Linear Time	0.00357	0.04123	298	0.09	0.9311
MGPS x Quadratic Time	0.04134	0.02719	298	1.52	0.1295
MGPS x Stress x Linear Time	-0.0254	0.06306	298	-0.4	0.6874
MGPS x Stress x Quadratic Time	**-0.1628**	**0.04234**	**298**	**-3.85**	**0.0001**

b. Sample 1. Individual Variant x Stress x Quadratic Time Interaction Terms, from Separate Models

	b	SE (b)	DF	t-value	p-value
HTR1A rs6295 G Alleles x Stress x Quadratic Time	-0.1757	0.06785	290	-2.59	0.0101
HTR2A rs6314C Alleles x Stress x Quadratic Time	-0.3530	0.12	300	-2.94	0.0035
TPH2 rs4570625 G Alleles x Stress x Quadratic Time	-0.0574	0.07329	280	-0.78	0.4343
5HTTLPR S-Carrier x Stress x Quadratic Time	-0.1075	0.1025	282	-1.05	0.2949

c. Sample 2. MGPS Primary Model

	b	SE (b)	DF	t-value	p-value
Intercept	0.9739	0.05825	356	16.72	< .0001
Linear Time	-0.0769	0.05913	118	-1.30	0.1961
Quadratic Time	-0.1764	0.03413	356	-5.17	< .0001
MGPS	-0.0038	0.06327	356	-0.06	0.9518
MGPS x Linear Time	0.0427	0.06418	356	0.67	0.5061
MGPS x Quadratic Time	-0.0627	0.03699	356	-1.70	0.0908

d. Sample 2. Individual Variant x Quadratic Time Interaction Terms, from Separate Models

	b	SE (b)	DF	t-value	p-value
HTR1A rs6295 G Alleles x Quadratic Time	-0.0702	0.04565	353	-1.54	0.1253
HTR2A rs6314C Alleles x Quadratic Time	-0.0608	0.09293	350	-0.65	0.5138
TPH2 rs4570625 G Alleles x Quadratic Time	-0.0086	0.05919	350	-0.14	0.8850
5HTTLPR S-Carrier x Quadratic Time	-0.0192	0.07237	344	-0.27	0.7911

produced significant results, HTR1A rs6295 and HTR2A rs6314, whereas TPH2 rs4570625 and 5HTTLPR produced nonsignificant individual results (interaction results Table 2b; full models, Supplement Table S4). Effect confidence intervals (95%), however, for TPH2 rs4570625 (−0.202 to 0.087) and 5HTTLPR (−0.309 to 0.094) overlapped those for HTR1A rs6295 (−0.309 to −0.042) and HTR2A rs6314 (−0.5890 to −0.117), suggesting that the SNP effects did not differ significantly in magnitude from one another.

3. Sample 2 and combined analyses

3.1. Methods

Because we observed effects of the serotonergic MGPS in the opposite direction as predicted (i.e., the MGPS predicted augmented not blunted reactivity to stress), we attempted to replicate findings in an independent sample. We conducted new genotyping in an existing German sample who completed a Socially Evaluative Cold Pressor Test and provided repeated salivary cortisol, described by Plieger et al. (2018). Further, the manipulation in Sample 2 was intermediate in its level of negative social evaluation between Sample 1′s control and explicit negative evaluative conditions, and used similarly timed cortisol sampling, permitting us to aggregate the datasets in a participant-level meta-analytic examination of the influence of negative evaluation level. We hypothesized that negative evaluation level would predict increasing MGPS effect size on cortisol reactivity.

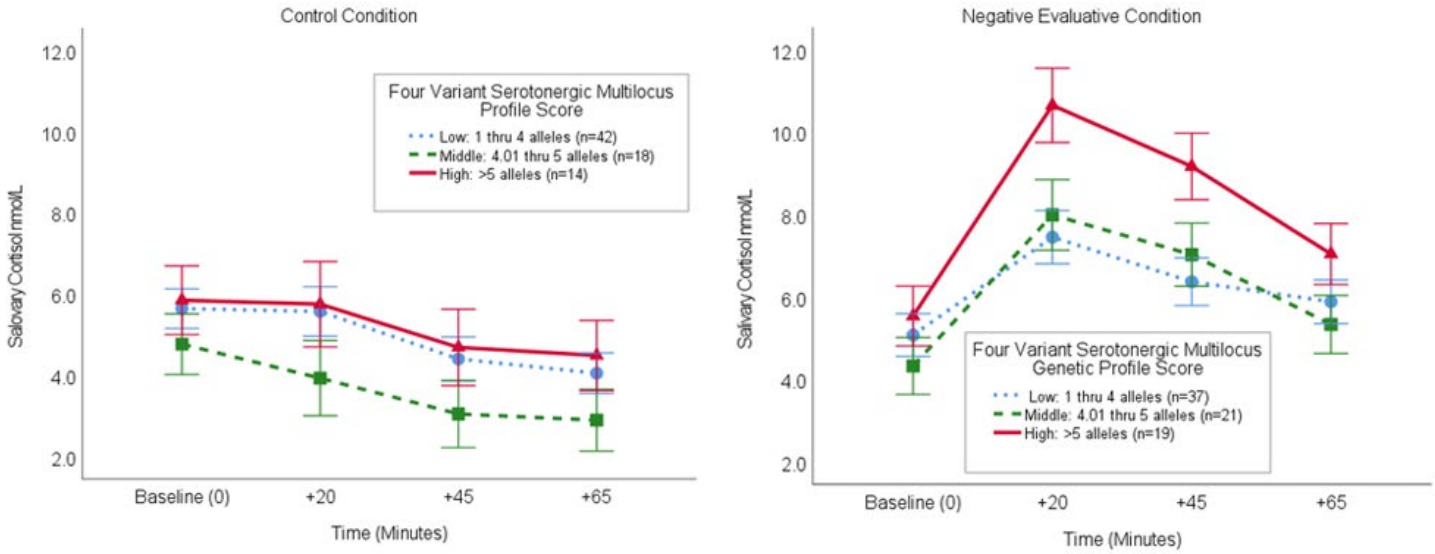

Fig. 1. Note: Sample 1 Cortisol Level as a Function of Stress Condition, Time, and Serotonergic Multilocus Genetic Profile Score. Data were analyzed as Ln Cortisol. Error bars represent +/1 SEM.

3.2. Participants

Sample 2 was all male (N = 128, ages 17–57) and was recruited at Bonn University (Germany) via online advertisements in social media networks, mailing lists, and postings throughout the University. No participants were familiar with laboratory stress protocols. The sample comprised almost exclusively White participants; no self-report ethnicity data were collected. All participants

provided informed, signed consent and were compensated with 15€. The local ethics committee approved the study.

3.3. Materials and procedures

Participants provided buccal swabs for DNA extraction. DNA was extracted using commercial MagNA Pure extraction kits (MagNA Pure LC DNA isolation kit; Roche Diagnostics; Mannheim, Germany). Two out of three SNPs (rs6314, rs6295) were genotyped using real-time PCR and a subsequent melting curve analysis. The third one (rs4570625) was genotyped by means of MALDI-TOF mass spectrometry using the MassARRAY-4 system (Agena Bioscience; Hamburg). Genotype frequencies were in HWE (all $\chi 2 < 1.56$, all $p > .18$). The genetic risk score was computed as described in Sample 1, permitting prorating for 1 missing genotype call ($n = 9$). This resulted in excluding $n = 3$ participants missing either 2 or 3 genotypes, for a final analytic sample of $N = 125$. MGPS values ranged from 3 to 7 ($M = 5.11$, $SD = .93$).

3.4. Stress manipulations and cortisol

Sample 2 completed the Socially Evaluative Cold Pressor Test (SECPT) in which participants were asked to immerse their non-dominant hand into iced water (Schwabe et al., 2008). They were asked to refrain from nicotine, caffeine, food intake, and other drinks except for water for forty-five minutes prior to the experiment. During the immersion, the experimenter pretended to intensely observe the participant and the participant was asked to look at a camera. All experimenters were female and not acquainted with the participants and provided no feedback conveying social support. Participants provided 5 saliva samples for cortisol: prior to the SECPT (and cognitive testing not reported here), i.e., baseline, immediately post-SECPT (+5 min after SECPT, which was +35 from baseline), + 20 min post-SECPT (+55 from baseline), and two further samples + 45 min and + 60 min post-SECPT (+75 and +90 min from baseline). To avoid confounds due to high morning cortisol levels, sessions were run from 3 pm to 5 pm and from 5 pm to 7 pm. For a more detailed description of the procedure, see Plieger et al. (2018).

3.5. Analytic plan

3.5.1. Sample 2

Growth curve modeling methods identical to Sample 1 were used, predicting the natural log of cortisol at 5 points. As in Sample 1, follow-up tests were conducted to examine the direction and magnitude of effect of individual SNPs in separate models and to examine covariates. A priori covariates selected to match those in Sample 1 (when possible) and to account for potential differences from Sample 1 were depression (either diagnosis or use of an antidepressant prescription, $n = 3$; coded no = 0, yes = 1), current smoking ($n = 15$; coded no = 0, yes = 1), age, and education level as an indicator of SES. As in Sample 1, all necessary constituent interaction terms (e.g., Covariate x MGPS) were included to partial effects of covariates out of the hypothesized interaction effect, per best practices (Keller, 2014).

3.5.2. Combined samples

In an effort to integrate results across the 2 samples, the samples were combined at the participant-data level to meta-analytically test the influence of negative evaluation level across samples. Although participant reports for perceived negative evaluation were not collected for Sample 2, the experimenter instructions regarding negative evaluation (i.e., an ambiguous level, cool and neutral) for Sample 2 approximate an intermediate condition used in prior research on three levels of negative evaluative TSSTs (none, intermediate, and explicit negative evaluation; Vrshek-Schallhorn et al., 2018). The final cortisol measure from Sample 2 was excluded from these analyses to approximately match the timing and number of the 4 samples collected in Sample 1, to permit combined growth curve analyses.2 The primary hypothesis of an effect of negative evaluative level predicting increased MGPS effects on cortisol reactivity was tested with the interaction Negative Evaluation Level × MGPS × Quadratic Time. Negative evaluation level was coded Sample 1 Control = 0, Sample 2 SECPT = 1, Sample 1 Challenge = 2. All dimensional effects were centered prior to analyses. Planned post-hoc tests in the event of significant findings included simple slope analyses for condition and two-group comparisons. Follow-up models probed whether results persisted when accounting for covariates available for both samples, age and gender.

3.6. Results

3.6.1. Sample 2 results

In the primary model for Sample 2, the Quadratic Time Effect (a simple effect at the mean of MPGS) supported that the manipulation provoked significant cortisol reactivity on average ($b = -0.176$, $SE(b) = 0.034$, $t(356) = -5.17$, $p < .0001$). Without covariates in the model, the effect of the MPGS x Quadratic Time approached significance, $b = -0.063$, $SE(b) = 0.037$, $t(356) = -1.70$, $p = 0.0908$ (Table 2c). With a priori covariates in the model for smoking, depression diagnosis or medication, age, and education as an indicator of SES, the effect of the MGPS approached significance, $b = -0.066$, $SE(b) = 0.037$, $t(352) = -1.78$, $p = 0.0754$ (Supplemental Table S5), such that higher MGPS corresponded to greater cortisol reactivity (Fig. 2), the same direction as Sample 1. Repeating the primary model with individual SNPs composing the MGPS, all effects were in the same direction, consistent with an additive effect, and none reached significance alone (summary effects, Table 2d; full models, Supplemental Table S6). Confidence intervals (95%) for all four SNPs were overlapping (TPH2 −0.125 to 0.108, HTR2A −0.244 to 0.122, HTR1A −0.160 to 0.020, and 5HTTLPR −0.162 to 0.123) suggesting their effects did not differ from each other.

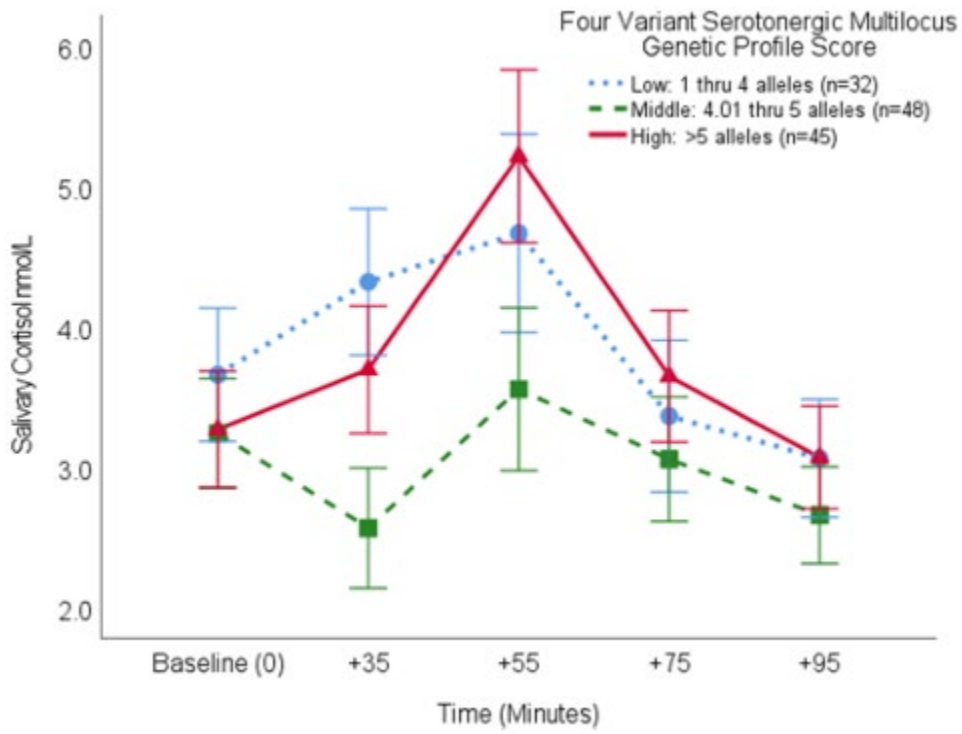

Fig. 2. Note: Sample 2 Cortisol Level as a Function of Time and Serotonergic Multilocus Genetic Profile Score. Data were analyzed as Ln-Cortisol. Error bars represent +/1 SEM.

3.6.2. Combined sample results

A participant level meta-analysis of the two samples combined probed the influence of the degree of negative evaluative threat (none in Sample 1′s Control, ambiguous/mild in Sample 2′s SECPT, explicit/robust in the Sample 1′s negative evaluative TSST) on the relationship between MGPS and cortisol reactivity. The MGPS × Negative Evaluation Level × Quadratic Time interaction effect was significant ($b = -0.083$, $SE(b) = 0.025$, $t(536) = -3.35$, $p = 0.0009$), and indicated that the effect of the MGPS on cortisol reactivity increased linearly as negative evaluation level of manipulation increased (Table 3). Simple slope effects (given by the MGPS × Quadratic Time interactions, from separate models that vary centering to identify each group's effect) probed whether the effect of this negative evaluation dimension was truly linear and not only driven by the original sample's negative evaluative TSST condition. Indeed, the MGPS effect size t-value on cortisol reactivity varied in an approximately linear manner (Sample 1 Control $t = 1.97$, $p = 0.0494$, Sample 2 SECPT $t = -1.37$, $p = 0.1706$, Sample 1 Challenge $t = -3.24$, $p = 0.0013$). Increasingly negative t-value magnitudes indicate that the MGPS effect on cortisol increasingly fits an inverted (negative sign) parabola indexing reactivity.

Strengthening this interpretation, in post-hoc two-condition analyses, Sample 2′s SECPT MGPS effect was more pronounced but not significantly different from the Sample 1′s control MGPS effect, $b = -0.0748$, $SE(b) = 0.04757$, $t(382) = -1.57$, $p = 0.1165$. Similarly, the Sample 1′s Challenge MGPS effect was more pronounced but not significantly different from Sample 2′s SECPT MGPS effect, $b = -0.0880$, $SE(b) = 0.055$, $t(388) = -1.59$, $p = 0.1120$ (Supplement Table S7). Consistent with the simple slope analyses across all three conditions, the effect sizes for these

two comparisons were almost identical (t = −1.57 versus t = −1.59), supporting that the MGPS's effects grew approximately linearly as negative evaluation increased.

Table 3. Participant level mini-meta-analysis results.

	b	SE (b)	DF	t-value	p-value
Intercept	1.3126	0.03709	536	35.39	< .0001
Linear Time	-0.04483	0.03156	267	-1.42	0.1566
Quadratic Time	-0.1382	0.02092	536	-6.61	< .0001
MGPS	-0.05641	0.03386	536	-1.67	0.0963
Negative Evaluative Level	0.1722	0.04995	536	3.45	0.0006
Negative Evaluative Level x Linear Time	0.1649	0.04367	536	3.78	0.0002
Negative Evaluative Level x Quadratic Time	-0.1689	0.02893	536	-5.84	< .0001
MGPS x Linear Time	0.03922	0.02903	536	1.35	0.1772
MGPS x Quadratic Time	-0.0264	0.01924	536	-1.37	0.1706
MGPS x Negative Evaluative Level x Linear Time	-0.00296	0.03748	536	-0.08	0.9371
MGPS x Negative Evaluative Level x Quad. Time	-0.0832	0.02483	536	-3.35	0.0009

Strengthening this interpretation, in post-hoc two-condition analyses, Sample 2′s SECPT MGPS effect was more pronounced but not significantly different from the Sample 1′s control MGPS effect, b = −0.0748, SE(b) = 0.04757, t(382) = −1.57, p = 0.1165. Similarly, the Sample 1′s Challenge MGPS effect was more pronounced but not significantly different from Sample 2′s SECPT MGPS effect, b = −0.0880, SE(b) = 0.055, t(388) = −1.59, p = 0.1120 (Supplement Table S7). Consistent with the simple slope analyses across all three conditions, the effect sizes for these two comparisons were almost identical (t = −1.57 versus t = −1.59), supporting that the MGPS's effects grew approximately linearly as negative evaluation increased.

A model covarying factors that differed in the sample designs indicated that the MGPS × Negative Evaluation Level × Quadratic time persisted when accounting for age and gender, b = −0.076, SE(b) = 0.025, t(529) = −3.03, p = 0.0026 (full model Supplement Table S8). Moreover, effects of research Sample (including the duration of the stress manipulation protocol) could not explain the observed effects of negative evaluation level on the MGPS-cortisol reactivity relationship (MGPS × Sample × Quadratic Time, b = 0.0095, SE(b) = 0.043, t(536) = 0.22, p = 0.8256, Supplement Table S9).

4. Integrative discussion

The present report provides the first documentation that a 4-variant MGPS predicts augmented cortisol reactivity to an explicitly negative evaluative lab-based stress induction, compared to a non-stressful control protocol. Although results from an independent sample utilizing a social evaluative cold pressor test only approached significance, a combined analysis of the three experimental conditions indicated that the MGPS effect size on cortisol reactivity grew significantly in a linear manner as the level of negative evaluation increased. This provides a conceptual replication of evidence that a serotonergic MGPS confers enhanced sensitivity to stress exposure, and it extends this evidence from a mental health outcome, depression diagnoses and symptoms, to a neuroendocrine intermediate outcome, cortisol reactivity. It further extends this work from naturalistic stress exposures to lab-administered controlled stress. The primary

implication for future investigation is that cortisol dysregulation may serve as an intermediate variable on the pathway from genetic sensitivity to depression onset.

4.1. Implications for understanding the "candidate environment"

The significant effect of negative evaluation level in the participant-level meta-analytic combined sample analyses echoes previous findings about the importance of identifying the environment most likely to evoke a genetic influence, i.e., the "candidate environment" (Vrshek-Schallhorn et al., 2014). An initial report showed that the 5-variant MGPS interacted with interpersonal stressful life events to predict depressive episodes and did not test non-interpersonal events (Vrshek-Schallhorn et al., 2015b), but critically, Starr et al., 2019 demonstrated that a GxE effect on depression symptoms was significantly larger for the MGPS and interpersonal stressful life events than non-interpersonal life events (Starr et al., 2019). The present results further suggest that the serotonergic MGPS is especially sensitive to interpersonal factors. However, taking a devil's advocate perspective, it also may be that the MGPS simply predicts more effectively the larger the average cortisol reactivity evoked by the experimental manipulation, and that varying negative evaluation is merely one way to achieve a larger cortisol output, but is not itself particularly important. Despite that other work supports that negative evaluative threat is particularly important to evoking larger cortisol responses (Woody et al., 2018), a decisive conclusion on the question of whether "more cortisol secretion" or "more negative evaluative threat" leads to greater MGPS prediction remains for replication efforts.

Other work has demonstrated the importance of stressor severity in real life and in lab-based stress: Major stressful life events out-perform minor events predicting depressive episode onset (Vrshek-Schallhorn et al., 2015a), and a lab-based study with three conditions (control, intermediate, explicitly negative evaluative) showed that cortisol reactivity significantly increases as negative evaluation level increases (Vrshek-Schallhorn et al., 2018). Taken together, the implication broadly for GxE is that GxE researchers should select the "candidate environment" with care, interpret GxE effects in light of the selected environment, and when possible, utilize stressors that are robust (or that can be modeled as dimensions of severity) and that account for interpersonal threat.

4.2. Implications for understanding cortisol reactivity

The direction of the MGPS effect in both samples—predicting augmented reactivity—was opposite our initial hypotheses for blunted reactivity. While contemporary views interpret both augmented and blunted reactivity as evidence of dysregulated HPA (Foley and Kirschbaum, 2010, McEwen, 1998), models have struggled to articulate why this is the case and what moderators predict which pattern will emerge. We have hypothesized that the level of negative evaluative threat severity moderates the relationship of depression risk factors, such as trait rumination (Vrshek-Schallhorn et al., 2018) or this MGPS, to cortisol reactivity, such that moderate severity (e.g., a standard TSST without explicit negative evaluation) would produce a positive association, whereas robust severity (e.g., an explicitly negative evaluative TSST such as used here) would produce a negative association.

This led us to an initial prediction that a serotonergic MGPS would, like trait rumination, predict blunted cortisol reactivity to negative evaluative stress, rather than the augmented reactivity found here; our error, however, may have been assuming that all "risk" factors will behave

similarly. On closer inspection, this MGPS and the previously studied risk factor, trait rumination, are likely to operate differently. This MGPS has been shown to conform to differential susceptibility theory, conferring sensitivity to the environment both for better and for worse. It is not likely to act on depression via main effect, but only to interact with life circumstances, yielding protective effects under good circumstances and deleterious effects under poor circumstances, which are likely to cancel each other out when main effects are examined without stress (such as would be the case in a genome-wide association study). Trait rumination, by contrast, is likely to predict greater depressive symptoms via main effect, and possibly also via an interaction effect with recent stress in which higher rumination amplifies the effects of stress. We would not expect trait rumination to intensify the good effects of a positive environment. Moreover, high trait rumination is likely to be skewed and concentrated among the poorest functioning individuals, whereas the MGPS is relatively normally distributed. Particularly in samples such as ours, unselected for pathology or prior stress exposure, higher MGPS is likely to reflect adaptive functioning on average, even as it may also indicate latent physiological sensitivity to prospective naturalistic interpersonal stressors.

We have previously conceptualized such adaptive functioning in cortisol responding to the negative evaluative TSST as augmented reactivity, viewing cortisol as a resource-mobilizing hormone, and the negative evaluative condition as one for which mobilization is appropriate. This view is also consistent with prior evidence that 5HTTLPR, for which there is robust evidence of differential susceptibility (Flasbeck et al., 2019, Sumner et al., 2015, Van IJzendoorn et al., 2012), predicts elevated cortisol reactivity in a meta-analysis (Miller et al., 2013), including to one explicitly negative evaluative manipulation (Way and Taylor, 2010). Taken together, this suggests a modification to the Cortisol Reactivity Threshold Model, such that risk factors operating via main effect on internalizing conditions will conform to the original model, whereas "sensitivity" factors operating via differential susceptibility on internalizing conditions will produce positive associations with cortisol reactivity regardless of level of negative evaluative threat, so long as the manipulation evokes a sufficient cortisol response on average.

4.3. Relationship to prior genetic work in cortisol

This work is one of the first examinations of additive or polygenic risk on cortisol reactivity to lab-induced stress, and the first using a candidate-based MGPS. In the only other two similar efforts to our knowledge, first, Pagliaccio et al. (2014) showed that an HPA-linked MGPS predicted greater cortisol increases to a series of standardized behavioral lab tasks in children. Second, following a genome-wide scan, Utge et al. (2018) linked a 6-variant polygenic score with cortisol reactivity and aspects of the diurnal rhythm in a sample of children. Importantly, however, variants that conform to differential susceptibility theory are not likely to be identified by genome-wide approaches such as Utge et al.'s in samples with normally distributed adversity backgrounds because the variants' associations with both protective and deleterious effects (as a function of better or worse circumstances) will cancel out (Zhang and Belsky, 2020). Thus, candidate-gene based additive MGPS scores offer utility.

Numerous reports, however, have previously linked single genetic variants to cortisol reactivity, including some in the serotonin system (Avery and Vrshek-Schallhorn, 2016, Brummett et al., 2014, Miller et al., 2013, Way et al., 2016). In particular, the present findings initially appear to contrast Armbruster et al.'s prior findings that the HTR1A rs6295 G allele predicts blunted cortisol reactivity (Armbruster et al., 2011). This SNP, however, presents challenges for comparing

across reports (when flanking sequences are unavailable) because it is ambiguously coded (i.e., C/G can denote either strand) and has a minor allele frequency near 50%. Thus, it is unverifiable whether the present results do in fact contrast Armbruster et al.'s or whether the reports have merely coded the gene in a reversed manner for analyses. To our knowledge, our report also provides the first evidence that HTR2A rs6314 predicts significantly differential cortisol reactivity. Taken together, this evidence supports the field's persistence in understanding genetic influence over cortisol reactivity.

4.4. Implications for future behavioral genetic work

These findings have several implications for future genetic research. First, none of the markers in the MGPS have been identified in GWAS analyses of cortisol reactivity (Utge et al., 2018) or depression (Wray et al., 2018). This may be either because this candidate gene work is entirely spurious as some argue (Border et al., 2019), or it may be because numerous variants for stress-reactivity and depression conform to differential susceptibility (Zhang and Belsky, 2020) and thus evade detection when background adversity is either ignored or improperly quantified, such as with measures that index stress perception rather than environmental exposure (Vrshek-Schallhorn et al., 2019a). Given conclusive evidence of the biological "significance," i.e., functionality, of several variants examined here (i.e., rs6295, rs6314, and 5HTTLPR, but not rs4570625, a meta-analytically indicated SNP; Lemonde et al., 2003; Ozaki et al., 1997), we argue that the behavioral genetic research community should give the latter possibility serious consideration. Second, because the findings did not appear to fully depend upon any one of the four SNPs—all contributed effects in the same direction and overlapped in their effect size confidence intervals in both samples—this work supports the future use of additive models, especially those located within a single neurobiological system that can then inform specific etiological pathways. Third, the findings support further investigation of HTR2A and its variant rs6314, because this interacted at a trend level with the cortisol awakening response to predict depression in recent work (Vrshek-Schallhorn et al., 2019b) and significantly predicted cortisol reactivity in these findings.

4.5. Implications for 5HT and HPA in depression etiology

The present results help inform our understanding of the pathway to depression at the neurochemical (i.e., hormonal, neurotransmitter/neuromodulator) level and raise novel possibilities. Evidence that the MGPS is associated with heightened cortisol production under lab-based stress—increasingly so as negative evaluative level grows—supports that heightened cortisol reactivity to interpersonal stress is one intermediate step in the pathway linking serotonergic genetic variability, interpersonal life stress exposure, and depression. Any explanation, however, must also be able to account for benefits that accrue to those with high MGPS levels under better conditions. Interestingly, whereas cortisol is often implicated in responding to negative social stimuli, it also plays a role in responding to positive stimuli. For example, an explicitly positive evaluative TSST version evoked cortisol responses that were descriptively larger even than those for an explicitly negative evaluative TSST version, and significantly larger than those for a neutral control TSST version (Taylor et al., 2010). Furthermore, in a study examining hourly cortisol and emotion over two consecutive weekdays, within-person elevations in cortisol predicted surges in alertness, active engagement, and even relaxation at the next self-report (Hoyt et al., 2016). Whether MGPS predicts heightened cortisol responses to

positive conditions remains an empirical question for future study. If higher serotonergic MGPS contributes to higher cortisol release under both positive and negative social conditions, this would suggest that cortisol is involved in both benefits and detriments associated with serotonin's role in differential susceptibility, and that environmental context substantially moderates the downstream neurobehavioral consequences of elevated cortisol. We can also cautiously infer that the observed effect of the MGPS on cortisol release under stress is due to altered synaptic serotonin levels in those with elevated versus lower MGPS. Although speculative, the differential susceptibility model suggests that serotonin levels may be more stable (i.e., insensitive to social conditions) in those with lower MGPS, and that serotonin levels might fluctuate more readily in those with elevated MGPS—conferring both the ability to flexibly modulate functioning to adapt to the social environment, but also the potential for surges in serotonin (and cortisol) levels to desensitize and downregulate downstream receptors.

Ultimately, however, a full explanation must span not only the genetic, hormonal and neurotransmitter/neuromodulator levels of analysis, but also the molecular, neurochemical, neural, behavioral, and emotional levels of analysis, under both positive and adverse social conditions. A recent review indeed highlights that the mechanisms involved in differential susceptibility are under-characterized, but also that serotonergic genetic variation potentially contributes to heightened salience network activity, and heightened connections between the salience network and two other networks—the default mode network and the central executive network connectivity (Homberg and Jagiellowicz, 2021). We echo their scientific call to action.

4.6. Limitations and future directions

Although the present work has several strengths, including a relatively novel genetic strategy, controlled stressors, repeated measurement of cortisol within subjects, and two independent samples, it is not without limitations. Data for rs1117899 could not be incorporated in Sample 1 due to problems with genotyping; future work in other samples should integrate this SNPs as well as others, perhaps including those in TPH1, for example (Piel et al., 2018). Similarly, we were only able to examine the influence of MGPS on one biomarker and on three conditions. Future work should investigate this and other MGPS in relation to additional intermediate outcomes, using interpersonal forms of stress (Vrshek-Schallhorn et al., 2015a).

4.7. Conclusions

We provide evidence that a 4-variant additive multilocus genetic profile score in the serotonin system predicts augmented cortisol reactivity to robustly stressful social, explicit negative evaluative versus non-stressful control lab conditions, and that this effect approached significance in a milder stress-induction protocol (the socially evaluative cold pressor test). Moreover, we report in combined analyses of the two samples that the effect of this genetic profile score significantly grows as negative evaluation level increases, in roughly equal increments with each increase in negative evaluation level. Because the 5- and 6-variant genetic score from which these four variants were drawn is associated with greater likelihood of depressive episode onset following major interpersonal stressful life events, the present results indicate that HPA-axis dysregulation may be one mechanism by which serotonergic genetic variation contributes to risk for depression.

5. Footnotes

1. Sensitivity analyses in Sample 1 addressed failures to conform to HWE in one marker included in the MGPS in the primary analyses, 5HTTLPR, and one marker not included in the MGPS in the primary analyses, HTR2C rs6318, due to lack of its availability in Sample 2. First, removing 5HTTLPR from the MGPS produced the same pattern of findings in Sample 1: MGPS (3 variant) x Stress x Quadratic Time b = −0.151, SE(b) = 0.044, t(298) = −3.39, p = 0.0008 (Supplemental Table S10a). Second, adding in the variant rs6318 (C-carrier, 0 = no, 1 = yes), which was not available in Sample 2, produced the same pattern of results in Sample 1: MGPS (5 variant) x Stress x Quadratic Time, b = −0.099, SE(b) = 0.039, t(296) = −2.56, p = 0.0109 (Supplemental Table S10b).

2. One could argue that in the mini meta-analysis, it would be appropriate to select Sample 2′s salivary cortisol samples 1, 3, 4, and 5 and exclude the second salivary cortisol sample for the combined analyses of two samples (rather than excluding the 5th and final cortisol sample), because a non-stressful cognitive task was administered immediately following the baseline saliva sample in Sample 2, prior to the SECPT, and the SECPT was briefer than the TSST. We anticipated this concern and re-ran the combined analysis using Sample 2′s salivary cortisol samples 1, 3, 4, and 5 in lieu of cortisol samples 1, 2, 3, and 4 used in the primary reported analyses. The overall pattern of results was virtually identical, including the effect of Negative Evaluation Level × MGPS × Quadratic Time, b = −0.084, SE(b) = 0.024, t(536) = −3.54, p = 0.0004, persistence of this effect after adding covariates, b = −0.075, SE(b) = 0.024, t(528) = −3.15, p = 0.0017, and lack of ability to substitute study site for Negative Evaluation Level, b = −0.025, SE(b) = 0.041, t(536) = −0.61, p = 0.5436. The exception, however, was that the simple effect of MGPS for the SECPT became significant in this iteration, b = −0.046, SE(b) = 0.018, t(536) = −2.49, p = 0.0130.

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Conflict of Interest

The authors declare no conflicts of interest.

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