

A Polymorphism in the Serotonin Transporter Gene Moderates Cardiovascular Reactivity to Psychosocial Stress

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Objective: To examine whether a polymorphism (5-HTTLPR: serotonin transporter linked polymorphic region) in the promoter of the serotonin transporter gene (*SLC6A4*) moderates cardiovascular reactivity to social threat. **Methods:** Psychologically healthy young adults delivered a speech and performed mental arithmetic in one of three conditions: a) an evaluative audience condition that gave disapproving and negative nonverbal social signals ($n = 59$); b) an evaluative audience condition that provided supportive social signals ($n = 60$); or c) a no audience condition ($n = 65$). Heart rate (HR) and systolic and diastolic blood pressures (DBP) were measured before, during, and after the stress tasks to assess cardiovascular reactivity and recovery. **Results:** In the negative audience condition, there was a significant association between the 5-HTTLPR and systolic blood pressure, DBP, and HR reactivity. Individuals with the short/short genotype showed the greatest reactivity. The DBP and HR reactivity of short/short individuals in the negative audience condition was also greater than that of individuals with the short/short genotype in the no audience condition. These associations of the 5-HTTLPR with HR reactivity were moderated by gender, being limited to females. With respect to cardiovascular recovery, short/short individuals in the negative audience condition exhibited impaired DBP recovery relative to other genotypes in the same condition, as well as short/short individuals in the no audience condition. **Conclusions:** The 5-HTTLPR moderates cardiovascular reactivity to stress in a threatening evaluative social context, which suggests that the serotonin system may be involved in the processes by which stressful, conflict-ridden social environments affect risk for cardiovascular-related health outcomes. **Key words:** serotonin, social, stress, trier, blood pressure, genetic variation.

5-HTTLPR = serotonin transporter gene linked polymorphic region; **CVR** = cardiovascular reactivity; **SBP** = systolic blood pressure; **DBP** = diastolic blood pressure; **HR** = heart rate.

INTRODUCTION

Over the last several decades, an accumulating body of evidence has established strong links between social factors and cardiovascular disease. For example, impoverished social connections are a predictor of coronary heart disease (1,2), and a lack of social support is associated with an increased likelihood of hypertension (3). The presence of conflict-filled relationships can also worsen the prognosis for heart disease, as shown in studies of strained marriages (4). Similarly, being raised in a threat-filled family environment ridden with frequent strife increases the probability of heart disease (5).

One possible means by which socially threatening environments may influence cardiovascular-related health outcomes is by influencing the extent to which the cardiovascular system, particularly blood pressure (BP), is activated by psychological stress (6). In prospective studies, greater cardiovascular reactivity (CVR) to stress has been linked with preclinical markers of coronary heart disease, such as coronary artery calcification (7) and carotid intima-media thickness (8). Further-

more, a recent meta-analysis (9) of prospective studies indicated that CVR is a significant predictor of higher resting systolic (SBP) and diastolic (DBP) blood pressures.

In line with such a model, growing up in a childhood environment characterized by frequent social conflict increases CVR to laboratory-based psychological stressors among adolescents (10,11) and young adults (12), although findings are not always consistent across genders. An environment of social conflict in adulthood has similar effects, as marital strife also increases CVR to psychological stressors in the laboratory (13). Insofar as CVR to adverse social environments is a risk factor for cardiovascular disease, it is important to better understand the biological processes affecting reactivity to social threats.

One factor that may be involved in this process is the neurotransmitter serotonin because pharmacological alteration of the serotonin system affects CVR to psychological stressors. In double-blind crossover studies with psychiatrically healthy subjects, chronic selective serotonin reuptake inhibitor (SSRI) treatment reduced CVR to psychological stress (14,15) compared with placebo. Similar results have been seen in studies (16–18) of clinical samples that did not use a crossover design. Conversely, reductions in central serotonin levels via tryptophan depletion increases CVR to psychological stress in patients with posttraumatic stress disorder successfully treated with an SSRI (19). Thus, it seems that augmenting central serotonergic neurotransmission decreases CVR, whereas reducing central serotonergic signaling increases CVR. This suggests that genetic variation in the serotonin system is likely to modulate CVR.

Within the promoter region of the serotonin transporter gene (*SLC6A4*), there is a polymorphism (serotonin transporter gene linked polymorphic region [5-HTTLPR]) that gives rise to two principal alleles: long and short. The long allele is associated with greater transcription in lymphocytes (20) and, consistent with the functional role of the serotonin transporter in serotonin reuptake, leads to greater serotonin uptake into platelets in some (21–23) but not all studies (24).

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In terms of central effects, the short allele is associated with reduced serotonergic neurotransmission (25–27), as assayed using a pharmacological challenge (prolactin release after alteration of serotonin transporter function). Because low levels of central serotonergic transmission are associated with risk factors for cardiovascular disease, such as elevated SBP and DBP (28), the metabolic syndrome (29), and carotid artery intima-media thickness (30), the short allele would be expected to be associated with greater CVR to stressors involving social threat.

Prior work (31) has found that, in a sample of healthy young European American adults, the short/short genotype was associated with greater heart rate (HR) reactivity to a psychological performance stressor (Stroop task and mental arithmetic), although the effect was limited to females. In contrast, in an older sample (32) of healthy African American and white participants, the long/long genotype was associated with greater HR and BP reactivity to the recall of an emotionally charged event in front of a small audience. These discrepant results suggest the need for further studies to clarify the nature of the relationship between the 5-HTTLPR and CVR, as well as identify potential variables that may moderate these effects. One such variable may be the degree to which a stressor invokes socially evaluative threat, as the presence of an evaluative audience can alter CVR (33). Recent work (34,35) indicated that the serotonin system affects sensitivity to both positive and negative social experiences and, therefore, may be particularly involved in responding to signals of social support and threat.

In the present study, this hypothesis was tested by having participants perform a speech and do mental arithmetic in front of a disapproving evaluative audience, a supportive evaluative audience, or a videocamera without an audience present. It was hypothesized that individuals with the short/short genotype would be most responsive to the negative, threatening social context, and they exhibit the highest CVR in this condition. It was expected that the positive social evaluation condition would elicit reduced reactivity relative to the negative audience condition, and the short/short genotype would be the most responsive to these differences in social context. Finally, it was anticipated that there would be the least CVR in the no audience condition without genotype dependent differences in CVR. As impaired cardiovascular recovery from psychological stressors is associated with adverse cardiovascular-related health outcomes (9), the relationship between the 5-HTTLPR and cardiovascular recovery was also assessed.

METHODS

Participants

Participants responded to a poster offering \$120 compensation for participation in the study. Prospective participants were screened during a telephone interview and were excluded from the study if: they were currently being treated by a mental health professional; they had mental or physical health problems (including posttraumatic stress disorder); or they were using mental health-related (e.g., SSRIs) or other medications that affect cardiovascular or endocrine function. In addition, pregnant or lactating women were excluded. All procedures were approved by the Institutional Review Board from the

University of California, Los Angeles. Data were collected between September 2006 and August 2008. The final sample consisted of 185 participants (39% male; 61% female; age range, 18–35 years). As participants were affiliated with the university as students, employees, or both, the sample reflects these demographics and was 37% Asian American, 22% European American, 16% Latino, 23% of “mixed” ethnicity, and 2% African American (these last three groups are designated as “other” in the analyses to be reported).

Procedure

Participants reported to the University’s Clinical Research Center in the mid to late afternoon. After arrival, participants were further screened to ensure that they had: 1) BP of <140/90 mm Hg; 2) a resting pulse between 60 beats/minute and 100 beats/minute; 3) normal lung auscultation; and 4) a normal cardiac examination (no evidence of congestive heart failure or arrhythmia). The nurse then inserted an indwelling catheter and obtained a blood sample to assay gonadal hormone and peptide levels (data not reported here). BP and HR were assessed every 3 minutes via a vital signs monitor (Dinamap 1846SX, Critikon, Inc., Tampa, Florida).

Each participant then took part in the Trier Social Stress Task, a widely used laboratory stress challenge known to elicit autonomic and hypothalamic-pituitary-adrenal axis stress responses (36). Participants were given 5 minutes to prepare a 5-minute speech on why they would be a good administrative assistant, a popular campus job for students and employees. They were then assigned randomly to deliver the speech in one of three audience conditions. In the no audience condition, participants delivered the speech only in front of the videocamera, which they were told was so that experts could later rate their performance. In both of the audience conditions, participants were told that they would not only be presenting in front of the videocamera for later evaluation but also in front of a live audience of trained evaluators who would rate their performance.

In the negative audience condition, the participant delivered his/her speech to two members of an evaluative panel who gave nonverbal indications of frustration over the quality of the speech. They displayed nonverbal signs of boredom and exchanged glances with each other that communicated mutual negative assessments. This manipulation represents a stronger version of the standardized audience condition for the Trier Social Stress Task. In the positive audience condition, the two audience members leaned forward, smiled, and gave nonverbal indications of approval. They occasionally exchanged glances with each other that communicated mutual positive assessments, and when not explicitly communicating positive assessments, their demeanor communicated interest in what the participant was saying. The two audience conditions mirrored each other precisely in terms of the timing and type of feedback, with the exception that the nonverbal feedback was positive in one condition and negative in the other (37). All panels included one man and one woman, and measures were taken to ensure the participant and audience members were unacquainted. The experimenter sat off to the side and out of direct view of the participant and did not give any verbal or nonverbal indications of positive or negative reactions to the participant’s speech performance.

Immediately after the speech, participants performed challenging mental arithmetic tasks for 5 minutes that required counting backward out loud by 7s and by 13s from 2,935, during which time they were urged by the experimenter to try to go faster. For the participants in the two audience conditions, these math problems were completed in front of the audience as well. After the mental arithmetic task, participants completed questionnaires. To assess the degree of recovery in the cardiovascular measures, BP and HR were measured 5 minutes after conclusion of the mental arithmetic task. At the conclusion of the experiment, the participant was debriefed and then dismissed.

Genotyping

Deoxyribonucleic acid (DNA) was collected from a saliva sample, using Oragene kits (DNA Genotek, Ottawa, Ontario, Canada) and extracted according to the manufacturer’s recommendations. The 5-HTTLPR was genotyped as described previously (38), using a modified protocol (20). The forward primer was 5′-GGC GTT GCC GCT CTG AAT GC-3′ (labeled with 6-car-

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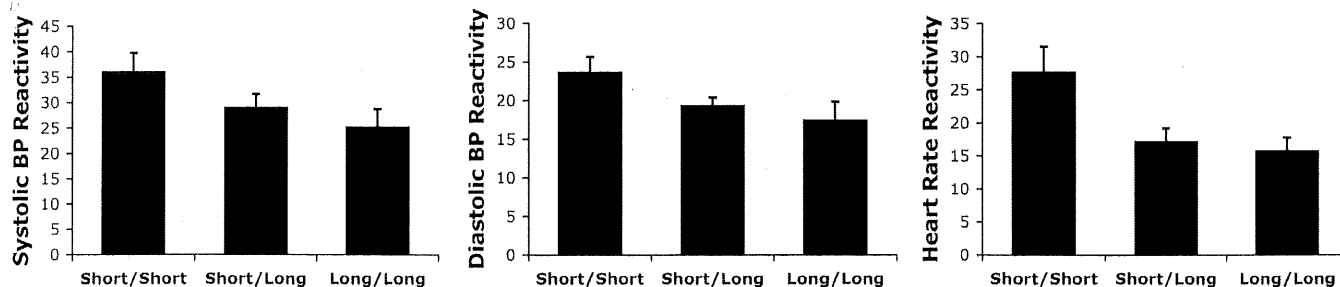


Figure 1. Relationship between the 5-HTTLPR (serotonin transporter linked polymorphic region) and systolic blood pressure reactivity (mm Hg), diastolic blood pressure reactivity (mm Hg), and heart rate reactivity (beats/minute) in the negative evaluative audience condition. Error bars denote standard error of the mean. BP = blood pressure.

boxyfluorescein fluorophore), and the reverse primer was 5'-GAG GGA CTG AGC TGG ACA ACC AC-3', which yielded 486-bp (short) and 529-bp (long) fragments. Polymerase chain reaction was performed in a total volume of 25 μ L, containing 100 ng of DNA, 160 nM of each primer, 1 mmol/L Tris-HCl (pH 8.3), 5 mmol/L KCl, 1.5 mmol/L MgCl₂, 2% DMSO (v/v), 2.5 U AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, California), 200 μ M of dATP, dCTP, dTTP, and 100 μ M of dGTP, and 7-deaza-2'-dGTP. After an initial denaturation at 94°C for 5 minutes, 35 cycles of denaturation (94°C for 30 seconds), annealing (63°C for 30 seconds), and extension (72°C for 1 minute) were performed followed by a final extension at 72°C for 20 minutes. The polymerase chain reaction products were electrophoresed on an ABI 3730 DNA analyzer (Applied Biosystems) with a Mapmaker size standard (Bioventures, Murfreesboro, Tennessee). We used GeneScan and Genotyper software (Applied Biosystems) for data collection and analysis.

Analyses

SBP, DBP, and HR reactivity were assessed by subtracting the baseline level of the respective measure from the average of the peak level during the speech stressor and mental arithmetic stressor (39). Recovery was assessed by subtracting the baseline level of each cardiovascular measure from the level of the measure 5 minutes after completion of the stress tasks. Tests of Hardy-Weinberg equilibrium for the entire sample and each ethnic grouping were conducted, using the software program Haploview v3.32 (<http://www.broad.mit.edu/mpg/haploview/>) (40). The relationship between the 5-HTTLPR and each cardiovascular dependent measure was assessed, using analyses of covariance. SPSS 17.0 (SPSS Inc., Chicago, Illinois) was used for all analyses, except for the three-way analysis of variance that used Stata 11.0 (College Station, Texas) with the "anovator" and "smcritical-value" programs (available at <http://www.ats.ucla.edu/stat/stata/ado/analysis/>). Baseline levels of each dependent measure were entered as a covariate in each analysis. For the assessment of genetic effects, two additional covariates for self-reported ethnicity (using two dummy variables: East Asians = 1, all others = 0; and European Americans = 1, all others = 0) were used to control for population stratification concerns. All statistical tests were two-tailed with α set to $p < .05$.¹

RESULTS

Descriptive Analyses

One participant could not be genotyped, leaving a sample of 184 participants. Across the three experimental conditions, there were no differences in the distribution of gender (χ^2 (2, $n = 184$) = 0.08, $p = .96$), ethnicity (χ^2 (4, $n = 184$) = 1.7, $p = .76$), or 5-HTTLPR genotype (χ^2 (4, $n = 184$) = 2.11, $p = .72$). Similarly, there were no differences between the groups in baseline SBP (F (2,181) = 0.097, $p = .91$), DBP

(F (2,181) = 0.002, $p = .99$), or HR (F (2,181) = 0.41, $p = .67$). Hardy-Weinberg equilibrium calculations showed no significant deviation from equilibrium for each ethnic grouping (all $p > .4$), as was the case for the calculation using the entire sample ($p = .19$).

Audience Effects on CVR

First, the effects of audience condition on CVR were assessed irrespective of genotype. There were significant effects of audience condition on SBP reactivity (F (2,180) = 8.31, $p < .001$, $\eta^2 = 0.085$), DBP reactivity (F (2,180) = 6.57, $p = .002$, $\eta^2 = 0.068$), and HR reactivity (F (2,181) = 9.16, $p < .001$, $\eta^2 = 0.09$). For each dependent measure, there were significant differences between the no audience condition and both the positive audience condition (SBP: F (1,180) = 12.83, $p < .001$; DBP: F (1,180) = 11.72, $p = .001$; HR: F (1,180) = 5.61, $p = .019$) and the negative audience condition (SBP: F (1,180) = 11.72, $p < .001$; DBP: F (1,180) = 7.67, $p = .006$; HR: F (1,180) = 18.05, $p < .001$), with reactivity higher in both audience conditions than in the no audience control. There were no significant differences in SBP or DBP reactivity between the two audience conditions ($p > .53$), whereas the HR reactivity differences approached significance (HR: F (1,180) = 3.46, $p = .065$). To determine if there was significant CVR in the control condition, a repeated-measures analysis of variance was used with baseline and peak reactivity as the within-subjects factor. There were robust increases in SBP (F (1,64) = 266.54, $p < .001$); DBP (F (1,64) = 457.07, $p < .001$), and HR (F (1,64) = 124.73, $p < .001$) in the absence of an audience.

5-HTTLPR and CVR to Psychological Stress

In the entire sample, there were no relationships between the 5-HTTLPR and baseline measures of SBP (F (2,179) = 0.05, $p = .95$), DBP (F (2,179) = 1.57, $p = .21$), or HR (F (2,179) = 0.004, $p = .996$). Because prior work (31,32) has examined the relationship between the 5-HTTLPR and CVR only in a negative stress condition, the effects of the 5-HTTLPR in only the negative audience condition were directly assessed to allow for comparisons with prior work.

According to a one-way analysis of covariance (using baseline value and ethnicity as the covariates), there was a significant main effect of the 5-HTTLPR on each of the CVR

¹The relationship between the 5-HTTLPR and cortisol responses to social stress in this sample have been published previously (41).

TABLE 1. Means and Standard Deviations (SD) for Each Genotype in the Different Audience Conditions

	SBP Reactivity (mm Hg)		DBP Reactivity (mm Hg)		HR Reactivity (beats/minute)	
	Mean	SD	Mean	SD	Mean	SD
No audience condition						
Short/short ($n = 27$)	23.07	10.00	16.85 ^b	6.46	11.15 ^b	07.37
Short/long ($n = 26$)	23.77	10.54	16.31	6.57	14.04	08.98
Long/long ($n = 11$)	21.45	16.91	19.18	6.01	11.18	12.43
Positive evaluative audience condition						
Short/short ($n = 22$)	31.73	9.01	21.91	6.34	15.68 ^b	7.70
Short/long ($n = 24$)	31.63	7.41	22.21	4.50	16.42	8.40
Long/long ($n = 13$)	28.77	12.15	18.46	7.34	19.62	9.19
Negative evaluative audience condition						
Short/short ($n = 18$)	36.17	15.10	23.78	8.14	27.78	15.78
Short/long ($n = 29$)	29.14 ^{a*}	13.47	19.48 ^a	5.15	17.28 ^a	10.06
Long/long ($n = 12$)	25.33 ^a	11.52	17.58 ^a	7.98	15.83 ^a	6.64

^a Significantly different from short/short genotype.

^b Significantly different from same genotype in negative audience condition.

* denotes marginally significant difference.

SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate.

dependent measures (Fig. 1): SBP reactivity: $F(2,53) = 3.25$, $p = .047$, $\eta^2 = 0.11$; DBP reactivity: $F(2,53) = 3.08$, $p = .054$, $\eta^2 = 0.1$; and HR reactivity: $F(2,53) = 5.94$, $p = .005$, $\eta^2 = 0.18$. Posttest comparisons revealed that, for each cardiovascular measure, the short/short genotype group had greater reactivity than the long/long genotype group (SBP reactivity: $F(1,53) = 5.99$, $p = .018$; DBP reactivity: $F(1,53) = 4.14$, $p = .027$; HR reactivity: $F(1,53) = 8.31$, $p = .006$), as well as the short/long group (SBP reactivity: $F(1,53) = 3.34$, $p = .073$; DBP reactivity: $F(1,53) = 4.07$, $p = .049$; HR reactivity: $F(1,53) = 9.34$, $p = .004$), whereas the differences between the short/long and long/long groups were not significant (all p values $> .27$).

To ensure that these significant effects in the negative audience condition were not due to population stratification, the relationship between the 5-HTTLPR and CVR was assessed separately for each ethnic grouping. Although the associations in this subdivided sample were not significant due to the reduced power, the qualitative pattern of reactivity was the same with the short/short individuals having the greatest SBP, DBP, and HR reactivity in each ethnic group (Supplemental Digital Content, <http://links.lww.com/PSYMED/A30>).

To assess the interactive effects of audience condition and genotype (Table 1), a two-way analysis of covariance was conducted for each dependent measure. For HR reactivity, there was a significant interaction between the 5-HTTLPR and audience condition ($F(4,172) = 4.13$, $p = .003$, $\eta^2 = 0.088$). Tests of simple effects revealed that the short/short individuals in the negative audience condition had significantly greater reactivity than the short/short individuals in the no audience condition ($F(1,172) = 29.81$, $p < .0001$) and the positive audience condition ($F(1,172) = 14.72$, $p < .001$).

For DBP reactivity, the interaction between genotype and audience condition approached significance ($F(4,172) = 2.13$, $p = .079$) with the short/short individuals in the negative

audience condition exhibiting greater reactivity than the short/short individuals in the no audience condition ($F(1,172) = 11.37$, $p < .001$), but not the positive audience condition ($F(1,172) = 0.78$, $p = .38$).

For SBP reactivity, there was not a significant interaction between audience condition and the 5-HTTLPR ($F(4,172) = 1.04$, NS).

Tests of Sex Differences

Because prior work (31) has found that the 5-HTTLPR can interact with sex to affect HR reactivity, we also assessed whether the effects of genotype and condition were moderated by sex. There was a significant three-way interaction between the 5-HTTLPR, sex, and audience condition ($F(4,163) = 3.71$, $p = .006$, $\eta^2 = 0.083$) for HR reactivity. Dividing by sex to decompose this interaction revealed that there was no interaction between audience condition and genotype ($F(4,163) = 0.44$, $p = .78$) for males. For females, there was a significant two-way interaction between genotype and audience condition ($F(4,163) = 7.61$, $p < .0001$). There was a significant main effect of the 5-HTTLPR in the negative audience condition ($F(2,163) = 17.35$, $p < .001$), but not in the no audience ($F(2,163) = 0.73$, $p = .582$) or positive audience condition ($F(2,163) = 0.54$, $p = .58$). In the negative audience condition (Fig. 2), females with the short/short genotype were significantly more reactive than females with the short/long genotype ($p < .05$) or long/long genotype ($p < .05$).

There was not a significant interaction between the 5-HTTLPR, sex, and audience condition for either SBP reactivity ($F(4,163) = 0.4$, $p = .81$) or DBP reactivity ($F(4,163) = 0.94$, $p = .44$).

5-HTTLPR and Cardiovascular Recovery

To assess the effects of audience condition and genotype on cardiovascular recovery, each respective cardiovascular mea-

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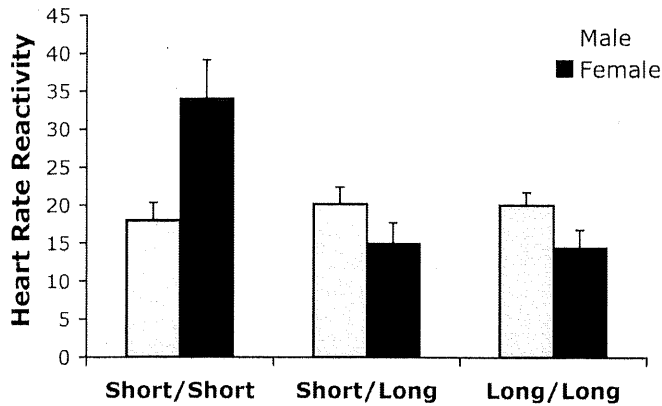


Figure 2. Relationship between the 5-HTTLPR (serotonin transporter gene linked polymorphic region) and heart rate reactivity (beats/minute) in the negative evaluative audience condition, separated by gender. Error bars denote standard error of the mean.

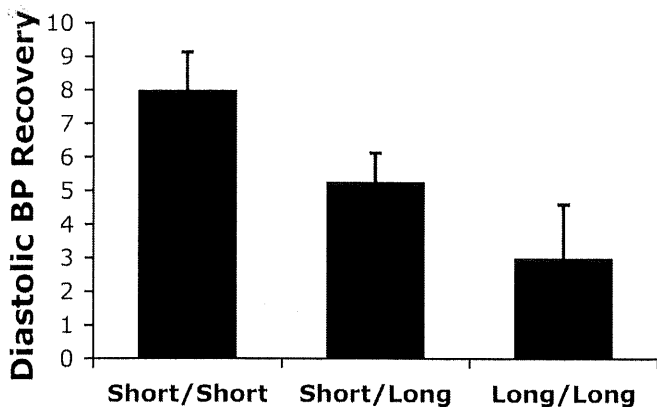


Figure 3. Relationship between the 5-HTTLPR (serotonin transporter linked polymorphic region) and diastolic blood pressure recovery (mm Hg) in the negative evaluative audience condition. Error bars denote standard error of the mean. BP = blood pressure.

sure 5 minutes after the termination of the stress task was subtracted from baseline levels before the task. There was not a significant effect of audience condition on DBP ($F(2,178) = 0.56, p = .57$) or HR recovery ($F(2,178) = 2.18, p = .12$), but there was an audience effect on SBP ($F(2,178) = 7.97, p < .0001, \eta^2 = 0.08$) recovery. In the positive audience condition, SBP remained higher than in the control condition ($F(1,180) = 14.59, p < .0001$) and the negative audience condition ($F(1,180) = 5.14, p = .025$).

With respect to the 5-HTTLPR, there was no interaction between genotype and audience condition for SBP recovery ($F(4,172) = 0.38, p = .82$) or HR recovery ($F(4,172) = 1.41, p = .23$). However, there was a significant interaction between the 5-HTTLPR and audience condition for DBP recovery (Fig. 3) ($F(4,172) = 3.42, p = .01, \eta^2 = 0.07$). Short/short individuals in the negative audience condition had significantly higher DBP than long/long individuals ($F(1,172) = 6.27, p = .013$) and short/long individuals ($F(1,172) = 3.13, p = .079$) in the same audience condition. Short/short individuals in the negative audience condition also had less recovery than the short/short individuals in the no audience ($F(1,172) = 4.27,$

$p = .04$) or positive audience ($F(1,172) = 5.71, p = .018$) conditions. The difference between the long/long individuals in the negative audience condition and the no audience condition approached significance ($F(1,172) = 3.47, p = .064$). All other comparisons were not significant (p values $> .28$).

With respect to sex differences, there was a significant main effect of sex on SBP recovery ($F(1,163) = 4.38, p = .038, \eta^2 = 0.03$; males: mean = 8.99, SD = 6.69; females: mean = 6.79, SD = 6.25). However, the interaction of sex, audience condition, and the 5-HTTLPR was not significant ($F(4,163) = 1.66, p = .16$). For DBP recovery and HR recovery, the main effect of sex was not significant ($p > .16$) nor were the three-way interactions ($p > .54$).

DISCUSSION

The results of this study demonstrate a significant relationship between the 5-HTTLPR and CVR to negative social evaluation, as predicted. Under conditions of high socially evaluative threat, there was a graded genotype-dependent relationship between the 5-HTTLPR and both SBP and DBP: Individuals with the short/short genotype were most reactive, followed by those with the short/long genotype, followed by those with the long/long genotype, who were the least reactive. The DBP reactivity of short/short individuals in the negative audience condition was significantly greater than the no audience condition. This heightened reactivity persisted 5 minutes after the stressor, as DBP levels of the short/short individuals in the negative audience condition remained significantly higher than those of short/short individuals in the no audience condition. The heightened reactivity to negative social evaluation of short/short individuals suggests that the 5-HTTLPR is particularly associated with the degree of reactivity to social threat, rather than just reactivity to stressors in general.

With respect to HR reactivity, a similar pattern was seen with the short/short genotype being especially reactive to negative social evaluation. The HR reactivity results were moderated by gender, however. Women showed significantly greater HR reactivity as a function of the 5-HTTLPR, whereas the results for HR reactivity were not significant for men.

In terms of mechanisms, the 5-HTTLPR-related differential reactivity to negative social cues could be a result of psychological factors operating at multiple stages in the information processing stream. At the initial stages of processing, the heightened response of short/short individuals to threatening and disapproving social signals is consistent with recent findings of a 5-HTTLPR-related attentional bias to threatening stimuli. In studies of attentional allocation using the dot-probe task with threatening words (41) or pictures (42), short allele carriers relative to long/long individuals have a negative bias, focusing greater attention on threatening stimuli and less on positive stimuli. In adolescents, a 5-HTTLPR short allele-dependent bias toward angry faces and away from happy faces has also been found (43). The degree of vigilance to socially threatening information, such as angry faces (44) or disapproving faces (45), has been found to be associated with

the cortisol response to psychological stress in the laboratory (44) and workplace (45). This bias reflecting greater attention to socially threatening stimuli may help explain the particular association of the 5-HTTLPR with CVR only in the negative evaluative audience condition, but not the positive evaluation condition or the no audience condition, where such social cues were not present.

The 5-HTTLPR related differential reactivity to the differing social cues could also be occurring at later stages of processing. Neuroimaging studies of social threat indicate that short allele carriers have greater amygdala reactivity to negative facial expressions than long/long individuals (46). As amygdala reactivity correlates with the degree of CVR to a stressor (47), it is likely that the amygdala is involved in the 5-HTTLPR-associated differences in CVR to the different experimental conditions in the present study.

In terms of later stages of processing, the 5-HTTLPR may also be associated with impaired emotion regulation capabilities. That the DBP of short/short individuals remained elevated after the negative social evaluation suggests that these individuals were less able to engage psychological processes that would effectively dampen this reactivity. Taken together, these data are consistent with the 5-HTTLPR affecting the degree of reactivity to social threats at multiple psychological levels.

The increase in HR reactivity in the negative audience condition was seen only in females with the short/short genotype, which is consistent with a prior study (31). Sex differences in HR reactivity are not without precedent, as an early meta-analysis (48) found that women responded to laboratory stressors with greater HR reactivity than men. Subsequent research examining the gender relevance of the stressor task as an explanation for such differences in CVR, including the social or nonsocial nature of the task, has led to mixed results (49,50). Based on the findings presented here, consideration of genotype in future studies may help to clarify some of these differences. The greater HR reactivity of short/short females is consistent with previously reported 5-HTTLPR-related sex differences using other dependent measures, which have shown that short/short females are more affected by stress than short/short males (51,52).

A prior study found that individuals with the long/long genotype had greater CVR to the recall of an emotional event (32), results that are potentially discrepant with the pattern in the current study and with McCaffery et al. (31). One potential resolution to this discrepancy is that the long allele adversely affects CVR via peripheral mechanisms, whereas the short allele increases CVR via central mechanisms (53). Insofar as aging affects peripheral cardiovascular processes associated with the 5-HTTLPR, such as platelet activation (54), the older age of the sample in the study by Williams et al. (32) may provide a potential explanation. In contrast, in the younger sample studied here, the 5-HTTLPR may be more associated with central responses to the stressor. Evidence for this supposition is that the 5-HTTLPR was also associated with cortisol reactivity in this sample (55), which is likely to be a

reflection of greater neural activation in response to the stressor. Ultimately, further studies with different psychological stress paradigms are needed to clarify the central versus peripheral roles of the 5-HTTLPR and serotonin system in modulating CVR.

Future research at the molecular level may also help to clarify the relationship between the serotonin transporter gene and CVR. Other polymorphisms in the SCL6A4 promoter (rs25531) (56), rs25532 (57), as well as 3' region (rs3813034) (58) have been demonstrated to affect SCL6A4 expression and may modulate the association between the 5-HTTLPR and CVR.

A limitation of this study is the heterogeneous ethnic composition of the sample, which raises concerns of potential population stratification artifacts due to the differences in allele frequency between ethnic groups (59). As one precautionary means to guard against such effects, self-reported ethnicity was entered as a covariate in the analyses (60). Furthermore, the qualitative relationship between the 5-HTTLPR and CVR was similar across the different ethnic groups, suggesting that the 5-HTTLPR was functioning similarly in each ethnic group. This is consistent with the vast majority of replicated gene-phenotype associations, which have been shown to be similar across ethnic groups (61):

CONCLUSION

The 5-HTTLPR moderates cardiovascular responsivity to stress marked by social threat and disapproval. These results are consistent with a growing body of literature, suggesting 5-HTTLPR involvement in setting sensitivity to the social environment (34) and reactivity to social threat in particular. The combination of 5-HTTLPR-related CVR seen in the present study, as well as cortisol reactivity seen in prior work (55), is indicative of the broad effects the serotonin system can have on multiple health-relevant physiological pathways. This widespread physiological influence of the serotonin system, in concert with its sensitivity to social experience, suggests that the serotonin system may be a critical link by which both interpersonal and societal level social factors influence health.

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