PsyCh Journal

INSTITUTE OF PSYCHOLOGY
Chinese Academy of Sciences

PsyCh Journal **8** (2019): 363–377
DOI: 10.1002/pchj.297

The serotonin transporter gene variants modulate acute stress-induced hippocampus and dorsomedial prefrontal cortex activity during memory retrieval

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Abstract: The short (s) allele of a polymorphism in the promoter region of the serotonin transporter gene (5-HTTLPR) is related to reduced serotonin transporter efficiency and an increased vulnerability to stress and mental disorders. In the present study, we investigated how 5-HTTLPR impacts on memory retrieval under stress and related neural activity by reanalyzing a small genetic neuroimaging data set. Twenty-seven healthy male volunteers participated in both the Trier Social Stress Test (TSST) and a respective control procedure and then their brain activity was measured with functional MRI (fMRI) while they performed an emotional-face-recognition task. Sixteen participants were carriers of the short allele (ss/sl carriers) and 11 were homozygous for the long allele (ll carriers). Genotype groups were compared with respect to stress-related physiological changes, memory performance, and brain activity. No significant genotype-dependent effects on memory performance or cortisol levels were found. The ss/sl carriers showed significantly higher systolic and diastolic blood pressure than the ll carriers, independent of stress. The ss/sl carriers reported stronger stress-induced nervous mood than the ll carriers. Our fMRI data revealed that the ss/sl carriers showed significantly weaker left hippocampus activation and stronger dorsomedial prefrontal cortex (dmPFC) deactivation when retrieving memories under stress as compared with the ll carriers. Subsequent analyses revealed that the distinct hippocampal activation pattern in both genotypes was associated with stress-induced cortisol elevation, while the distinct dmPFC activation pattern in both genotypes was associated with stress-induced changes in reaction times. Our results thus add new evidence that serotonin signaling modulates neural activity in the hippocampus and dmPFC during memory retrieval under acute psychosocial stress.

Keywords: acute stress; dorsomedial prefrontal cortex (dmPFC); hippocampus; 5-HTTLPRmemory retrieval

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Received 30 November 2018. Accepted 10 April 2019.

When confronted with a changing and stressful environment, humans show different levels of vulnerability to the stressful events and this vulnerability is codetermined by nature (such as genes) and nurture (such as learning experiences). For decades, a large body of research on the serotonin transporter (5-HTT) gene promotor polymorphism (serotonin-transporter-linked polymorphic region [5-HTTLPR]) has provided more and more evidence on gene-by-environment interactions.

It is generally accepted that individuals with one or two copies of the short (s) allele (ss/sl carriers) of 5-HTTLPR are more sensitive to stressful events than those homozygous for the long (l) allele (ll carriers), and are more susceptible to stress-related mental disorders (Caspi et al., 2003). However, two meta-analyses in the last year have shown contradictory findings. Culverhouse et al. (2018) combined 31 data sets containing 38,802 European-ancestry participants and assessed for 5-HTTLPR genotypes, depression, and stressful life events, such as childhood maltreatment, and found no genotype impact on depression risk. In contrast, Bleys, Luyten, Soenens, and Claes (2018) included 51 relevant studies containing 51,449 participants and found that the overall effect size of gene-by-environment effect was significant, therefore supporting the interaction of 5-HTTLPR with stress in predicting depression. The two studies used different data sets and a different analysis approach; for instance, Culverhouse's study reported only European-ancestry participants and analyzed the sex effects and such factors were not discussed in Bleys's study. Given the fact that stress events happen during an individual's life in an unpredictable and uncontrollable manner, Culverhouse and colleagues suggest: "If an interaction exists in which the S allele of 5-HTTLPR increases risk of depression only in stressed individuals, then it is not a broadly generalizable effect, but must be of modest effect size and only observable in limited situations" (Culverhouse et al., 2018, p. 134).

Therefore, it is necessary to limit studies to a highly controlled laboratory situation and investigate the underlying neurobiological mechanisms. Previous functional MRI (fMRI) studies provide evidence that the genotype-linked vulnerability towards psychiatric disorders might relate to amygdala function. For example, ss/sl carriers show stronger right amygdala and right fusiform gyrus activity when processing negative facial stimuli (Hariri et al., 2002, 2005), stronger right amygdala activity when processing negative words (Canli et al., 2005), and a stronger positive coupling between the bilateral amygdala and the ventromedial prefrontal cortex when processing affectively aversive versus neutral pictures (Heinz et al., 2005). Hariri et al. (2002) proposed that in ss/sl carriers the heightened amygdala activity is related to a relatively decreased 5-HT expression and increased available synaptic 5-HT acting on excitatory 5-HT receptor subtypes. Another fMRI study found that s-homozygous participants felt more distressed when recalling negative traits of themselves as compared to recalling those traits of their friends, which was related to increased neural activity in the dorsomedial prefrontal cortex (dmPFC) and anterior insula (Ma et al., 2014).

Most of the previous studies investigating the 5-HTTLPR genotype effect used either questionnaires or emotional stimuli simulating a threatening situation, but rarely used a socially stressful condition. Mueller et al. (2011) used the Trier Social Stress Test (TSST) to induce acute psychosocial stress and found that in younger adults, the ll carriers showed a stronger TSST-induced cortisol response than the ss/sl carriers. However, another study (Way & Taylor, 2010) and a recent meta-analysis (Miller, Wankerl, Stalder, Kirschbaum, & Alexander, 2013) revealed contradictory findings, suggesting that s-homozygous participants exhibit larger cortisol reactivity to acute stress. In a recent study, Williams and colleagues (2017) reported that carriers of one or two l alleles had a higher systolic blood pressure (SBP) and a higher risk of severe hypertension. This is in line with earlier findings that the l allele is associated with increased cardiovascular reactivity (including SBP, diastolic blood pressure [DBP], and heart rate [HR]) to emotional recall (Williams et al., 2001, 2008), stronger anxiety symptoms, and more frequent stressful life events (Ming et al., 2015). Hence, findings on the role of the 5-HTTLPR genotype in physiological responsiveness to stressful events are equivocal. Although s-allele carriers may have a larger 5-HT availability and therefore may be more vulnerable to stressful events and negative emotions, l-allele carriers may be predisposed to increased cardiovascular responses to acute stress (Williams et al., 2017). Based on these facts, it is still too early to simply conclude that only the s-allele carriers are sensitive to stress, as many of the previous studies have proposed (Canli et al., 2005; Caspi et al., 2003; Hariri et al., 2005; Heinz et al., 2005).

Accumulating evidence suggests that stress exposure during development can induce chronic stimulation of the hypothalamic–pituitary–adrenal (HPA) axis and lead to modifications in the consequent reactivity to acute stressors, therefore influencing the quality and quantity of memory processes (Fink, 2016; Schwabe, Wolf, & Oitzl, 2010). Such impact on memory may have deleterious implications for several mental disorders, especially post-traumatic stress disorders (PTSD; Nader Amir & Bomyea, 2010; Nemeroff et al., 2006), major depressive disorders (MDD; Disner, Beevers, Haigh, & Beck, 2011; McEwen, 2004; Whalley, Rugg, Smith, Dolan, & Brewin, 2009; Wingenfeld & Wolf, 2011), and generalized anxiety disorder (Kalueff, 2007). PTSD symptoms often involve involuntary

retrieval of distressing memories (flashbacks) and are associated with reduced activity in the medial prefrontal cortex (mPFC) and anterior cingulate cortex (ACC; Bremner et al., 1999; Lanius et al., 2001). Such abnormal memory retrieval might relate to the modulatory influence of the stress hormone cortisol or the neurotransmitter serotonin (Riedel, Sobczak, Nicolson, & Honig, 2002). Riedel and colleagues found that stress-induced cortisol response was negatively associated with delayed verbal memory recall and recognition, and acute tryptophan (the 5-HT synthesis precursor) depletion blocked such association in both first-degree relatives of bipolar patients and healthy matched controls (Riedel et al., 2002). Later studies found that acute tryptophan depletion impaired word recall independent of valence (Roiser, Cook, Cooper, Rubinsztein, & Sahakian, 2005) or for positive words only (Firk & Markus, 2009) in 5-HTTLPR s-homozygous subjects.

In order to determine whether 5-HTTLPR genotype affects memory retrieval under acute psychosocial stress, we reanalyzed a previously published data set (Li, Weerda, Milde, Wolf, & Thiel, 2015) and included the 5-HTTLPR genotype as a new independent variable, in order to identify how serotonergic signaling impacts brain activity during memory retrieval under acute psychosocial stress. We hypothesized that the ss/sl carriers would show impaired memory retrieval and a differential pattern of acute-stress-related brain activity pattern (especially in the hippocampus and dmPFC) when compared with the ll carriers.

Materials and methods

Participants

Data of 27 young healthy men (average age of 24.25 ± 0.75 years; average body mass index [BMI] of 22.78 ± 0.387 kg/m^2) were included in the current analysis. None of them suffered from any acute or chronic disease or took medication. The study was originally approved by the ethics committee of the University of Oldenburg, and all participants provided written informed consent.

Procedure

The psychosocial stress protocol with an emotional-face-recognition task was previously used in Li, Weerda, Milde, Wolf, and Thiel (2014) and Li et al. (2015). Since this is a within-subject design, all 27 participants complete both stress and control TSST. The Stress-TSST included a 2-min introduction, 3 min of preparation, 5 min of a highly controlled job interview in front of a "stress committee" and a video camera, and 5 min of an arithmetic task. The Control-TSST included a 2-min introduction, 3 min of preparation, 5 min of a self-description of their last journey (without an audience or camera), and 5 min of a rather easy arithmetic task. The experiment lasted in total 170 min, with 20 min for the encoding phase, a 60-min break, 15 min for the TSST or respective control procedure (Het, Rohleder, Schoofs, Kirschbaum, & Wolf, 2009; Kirschbaum, Pirke, & Hellhammer, 1993; Li et al., 2014), followed by 30 min for the retrieval phase. The genetic probe was collected (see below) at the beginning of the experiment. In addition, we collected the participants' saliva for later analysis of salivary cortisol, blood pressure, and mood self-rating questionnaires at three time points (before TSST, after TSST, and after memory retrieval) to make sure that the stress was successfully induced. Stress and control sessions were separated by 1 week ($\pm$1 day), and the order of the stress and control conditions was randomized across participants. Twenty-seven healthy male volunteers participated in both the Trier Social Stress Test (TSST) and a respective control procedure and then their brain activity was measured with functional MRI (fMRI) while they performed an emotional-face-recognition task. The face stimuli used in both conditions included 100 faces during encoding (half fearful and half neutral emotion) and another 50 during retrieval (half fearful and half neutral emotion), and the pictures were counterbalanced with respect to previously rated emotionality, arousal, and picture quality, as well as the gender of the faces. For further description of the stimuli and the emotional-face-recognition task, see Li et al. (2014). We analyzed accuracy and reaction times of correct answers during recognition using mixed-design analysis of variance (ANOVAs), with the within-subject factors of stress condition (control/stress) and emotion (fearful/neutral) and the between-subject factor of genotype (ss/sl carriers). Significant effects were followed by post-hoc *t* tests. All statistical analyses of the behavioral data were run using SPSS 18.0 (SPSS GmbH, Munich, Germany).

Cortisol, cardiovascular reactivity, and subjective measures

Salivary cortisol concentrations, blood pressure (SBP and DBP), pulse, and subjective mood ratings were collected at four time points: (1) prior to the encoding phase, (2) prior

to the TSST/control condition, (3) directly afterwards, and (4) after completion of the retrieval session (i.e., approx. 40 min after TSST). Saliva was collected using Salivette collection devices (Sarstedt, Nümbrecht, Germany), which were stored afterwards at −20 °C until analysis. Biochemical analysis was performed by the lab of Professor C. Kirschbaum, Dresden, Germany: Salivary cortisol levels were assessed using a luminescence immunoassay (IBL GmbH, Hamburg, Germany). Inter- and intra-assay variations were below 10%. As the cortisol values of two participants could not be determined due to technical problems in one of the sessions, we ran cortisol analyses on the data of 25 participants only.

Affective responses were assessed with the German version of the Multidimensional Mood State Questionnaire (Steyer, Schwenkmezger, Notz, & Eid, 1994) after collection of saliva samples. The questionnaire consists of 24 items, each with a 5-point rating scale. Three underlying dimensions were calculated based on these 24 items: Good Mood–Bad Mood, Alertness–Tiredness, and Calmness–Nervousness.

Physiological and mood effects were analyzed using mixed-design ANOVAs, with the main effects of time (before TSST, after TSST, and after scanning), stress condition (control/stress), and genotype (ss/sl carriers/ll carriers). Significant effects were followed by post-hoc *t* tests using SPSS 18.0.

Genotyping

DNA was extracted from oral epithelium cells and genotyping was performed according to the Institute for Polymorphism and Mutation Analysis, Homburg, Germany (Heils et al., 1996; Nakamura, Ueno, Sano, & Tanabe, 2000). Sixteen participants who carried the short allele were categorized as the ss/sl carriers (including 14 s-homozygous and two heterozygous participants), and the other 11 participants who carried only the long allele were categorized as the ll carriers. We calculated the Hardy–Weinberg equilibrium and there is significant statistical support that the population is not in Hardy–Weinberg equilibrium, due to only two heterozygous participants within the whole sample. Previous studies have suggested that carriers of the 5-HTT short variant have less serotonin uptake than ll homozygous subjects (Canli et al., 2005), and in the present study the homozygous subjects (ss and ll) were relatively balanced (14 vs. 11) and the group sizes of homozygous subjects were close to those of the above-mentioned reference. Therefore, we think it is reasonable to report our findings based on our current data. There were no differences with respect to the order effect between groups: in the ss/sl carriers, nine participants experienced the stress condition first, and seven participants experienced the control condition first; while in ll carriers, seven participants experienced the stress condition first, and four participants experienced the control condition first.

MRI neuroimaging

A 1.5-T Siemens MAGNETOM Sonata MRI system with an eight-channel head coil was used to obtain T2*-weighted gradient echo planar imaging volumes with blood oxygenation level-dependent (BOLD) contrast (for MRI setting details, see Li et al., 2014). The functional data were originally pre-processed and modeled at single-subject level in SPM8 (FIL, Wellcome Trust Centre for Neuroimaging, UCL, London, UK) for the encoding and retrieval phase (for details, see Li et al., 2014).

Single-subject data were originally modeled with 16 regressors of interest modeling the effects of neutral and fearful faces in the stress and control conditions as a function of novelty and correctness (e.g., control condition: fearful old face that was correctly recognized; control condition: fearful old face that yielded an incorrect answer; control condition: fearful new face that was correctly recognized). In order to be consistent with previous analyses of this data set, we used the previously processed single-subject data of the retrieval phase, which was subjected to a genotype-dependent second-level analysis using SPM12. To investigate genotype-dependent stress effects, a weighted contrast coding for BOLD signal increases to the main effect of stress (stress greater than control [stress > control] pooling over all face conditions in the retrieval phase) was computed and entered into a paired *t* test at the second level to assess stress-induced differences between the two genotypes. Results were reported at cluster-level $p \leq .05$ family-wise error (FWE) correction for voxels surpassing a $p \leq .001$ initial voxel threshold. To illustrate significant effects, mean beta values were extracted from significant clusters using the Marsbar toolbox (Brett, Anton, Valabregue, & Poline, 2002).

Correlation analysis between BOLD signal and behavior/cortisol data

To investigate whether genotype-dependent brain activity during memory retrieval under stress relates to behavior or

physiological reactivity, we performed further regression analyses to investigate the relation between changes in BOLD activity and changes in accuracy, and speed of correct responses as well as stress-induced changes in cortisol. To examine the effect of stress on behavioral performance, we first calculated the TSST-induced change in accuracy and reaction time (RT) for all correct responses; for example, $\Delta ACC = ACC_{stress} - ACC_{control}$, where ACC_{stress} stands for the accuracy under stress condition and $ACC_{control}$ stands for the accuracy under control condition; and $\Delta RT = RT_{stress} - RT_{control}$, where RT_{stress} stands for the RT under the stress condition and $RT_{control}$ stands for the RT under the control condition. Second, we calculated the TSST-induced cortisol elevation; for example, ${}^{stress}TSST_{diff} = {}^{stress}TSST_{after} - {}^{stress}TSST_{before}$, where $TSST_{after}$ stands for the cortisol concentration after TSST treatment and $TSST_{before}$ stands for the cortisol concentration before TSST treatment. Then we calculated the condition difference of $\Delta TSST_{diff}$: $\Delta TSST_{diff} = {}^{stress}TSST_{diff} - {}^{control}TSST_{diff}$ where ${}^{stress}TSST_{diff}$ stands for the TSST-induced cortisol elevation under the stress condition and ${}^{control}TSST_{diff}$ stands for the TSST-induced cortisol elevation under the control condition. Pearson's correlation was calculated to study the relationship between stress-induced differences in brain activity (mean betas of the significantly activated clusters under stress vs. control) and the stress-induced behavior/cortisol change (ΔACC, ΔRT, $\Delta TSST_{diff}$) for both genotypes. In order to compare the significance of the difference between the correlation coefficient in the ll carriers and ss/sl carriers, we used the Fisher *r*-to-*z* transformation that calculated the value of *z*, and calculated the correlation coefficients' significance. The correlations in the ll carriers and ss/sl carriers were considered as significantly different when $p \leq .05$ (two-tailed). Two participants were not included in the $\Delta TSST_{diff}$ calculation due to cortisol data missing, as described above.

All statistical analyses were performed using SPSS 18.0 (SPSS GmbH, Munich, Germany). The two correlation coefficients' differences were calculated using the website VassarStats (http://vassarstats.net/rdiff.html).

Results

Genotype-dependent stress response

In the stress condition, although the cortisol concentration increased significantly after TSST (see Li et al., 2014), there was no main effect of genotype, $F(1, 23) = 0.14$, $p = .70$, nor any interactions: genotype-by-stress interaction, $F(1, 23) = 0.18$, $p = 0.96$; genotype-by-time interaction, $F(2, 46) = 1.20$, $p = .31$; and genotype-by-stress-by-time interaction $F(2, 46) = 0.28$, $p = .76$. Descriptive statistics of the cortisol concentration in each group/condition at each time point are reported in Table 1.

Similarly, the SBP and DBP increased significantly after the TSST in the stress condition (see Li et al., 2014), and there was a significant main effect of group for SBP, $F(1, 25) = 4.66$, $p < .05$, and DBP, $F(1, 25) = 4.99$, $p < .05$, with higher values in ss/sl carriers than ll carriers. Moreover, the DBP showed a significant genotype-by-time interaction, $F(2, 50) = 3.30$, $p < .05$. A post-hoc *t* test showed that the ss/sl carriers showed significantly higher DBP after TSST, $t(25) = 2.50$, $p < .05$, and after memory retrieval, $t(25) = 2.57$, $p < .05$, than the ll carriers (see Figure 1). There was no genotype-by-stress interaction—SBP: $F(1, 25) = 1.32$, $p = .26$; DBP: $F(1, 25) = .69$, $p = .41$; no genotype-by-time interaction for SBP—$F(2, 50) = 1.94$, $p = .15$; and no genotype-by-stress-by-time interaction—SBP: $F(2, 50) = .33$, $p = .72$; DBP: $F(2, 50) = .45$, $p = .64$. There were no significant effects with respect to the pulse data—main effect of genotype, $F(1, 25) = 0.07$, $p = .80$; genotype-by-stress interaction, $F(1, 25) = 2.46$, $p = .13$; genotype-by-time interaction, $F(2, 50) = 0.75$, $p = .48$; and genotype-by-stress-by-time interaction, $F(2, 50) = 0.73$, $p = .49$. Descriptive statistics of the SBP, DBP, and pulse in each group/condition at each time point are reported in Table 1.

For the Good Mood–Bad Mood (GB), Alertness–Tiredness (AT), and Calmness–Nervousness (CN) mood scales, only the GB and CN showed a significant stress effect, as previously reported in Li et al. (2014). There was a significant genotype-by-stress interaction for the CN scale, $F(2, 50) = 4.78$, $p < .05$: the ss/sl carriers rated themselves to be more nervous than the ll carriers under stress—ll carriers: $t(10) = 3.13$, $p < .05$; and ss/sl carriers: $t(15) = 5.92$, $p < .001$ (see Figure 2). For the CN scale, there was no significant main effect of genotype, $F(1, 25) = 1.71$, $p = .20$; of genotype-by-time interaction, $F(2, 50) = 2.48$, $p = .09$; or of genotype-by-stress-by-time interaction, $F(2, 50) = 0.14$, $p = .87$. For the GB and AT scales, no genotype-dependent effects were found: main effect, GB: $F(1, 25) = 0.26$, $p = .61$; AT: $F(1, 25) = 0.10$, $p = .75$; genotype-by-stress interaction, GB: $F(1, 25) = 0.95$, $p = .34$; AT: $F(1, 25) = 0.28$, $p = .60$;

Table 1
Stress responses in each genotype group

	Condition	Group	Before TSST/control (mean ± SE)	After TSST/control (mean ± *SE*)	After retrieval (mean ± *SE*)
Cortisol (nmol/l)	Control	ll carriers	10.91 ± 2.46	10.64 ± 1.78	8.62 ± 1.61
		ss/sl carriers	6.64 ± 0.98	9.19 ± 2.01	7.09 ± 1.42
	Stress	ll carriers	9.34 ± 2.54	15.46 ± 2.19	12.25 ± 1.35
		ss/sl carriers	9.24 ± 2.37	16.52 ± 2.60	14.60 ± 3.22
Systolic blood pressure (mmHg)	Control	ll carriers	115.18 ± 4.63	113.27 ± 4.19	111.45 ± 3.86
		ss/sl carriers	121.25 ± 2.74	128.25 ± 4.05	123.50 ± 2.86
	Stress	ll carriers	114.18 ± 4.54	124.18 ± 4.38	120.18 ± 3.57
		ss/sl carriers	119.31 ± 2.52	132.19 ± 4.03	128.00 ± 3.48
Diastolic blood pressure (mmHg)	Control	ll carriers	72.64 ± 5.04	68.45 ± 2.86	69.27 ± 2.90
		ss/sl carriers	75.50 ± 2.17	81.69 ± 3.65	77.94 ± 1.78
	Stress	ll carriers	71.09 ± 4.27	76.82 ± 3.48	74.36 ± 2.04
		ss/sl carriers	74.00 ± 1.93	84.50 ± 2.68	81.69 ± 2.64
Pulse (beats per minute)	Control	ll carriers	60.64 ± 2.05	59.82 ± 2.01	61.00 ± 2.47
		ss/sl carriers	63.44 ± 1.78	63.88 ± 2.42	63.31 ± 1.94
	Stress	ll carriers	65.27 ± 2.62	67.36 ± 3.60	63.73 ± 2.75
		ss/sl carriers	60.87 ± 1.60	67.44 ± 2.70	62.75 ± 2.18
Good–Bad Mood	Control	ll carriers	34.64 ± 1.36	35.00 ± 1.31	34.73 ± 1.41
		ss/sl carriers	33.94 ± 1.00	34.88 ± 1.14	35.56 ± 1.04
	Stress	ll carriers	34.55 ± 1.12	28.36 ± 2.09	33.27 ± 1.38
		ss/sl carriers	34.88 ± 0.97	25.69 ± 1.70	30.75 ± 1.79
Alertness–Tiredness	Control	ll carriers	26.91 ± 1.64	31.27 ± 1.23	26.91 ± 1.91
		ss/sl carriers	28.94 ± 1.63	31.69 ± 1.19	27.38 ± 1.62
	Stress	ll carriers	28.55 ± 1.23	29.73 ± 1.73	27.09 ± 2.01
		ss/sl carriers	29.06 ± 1.34	30.25 ± 1.45	26.69 ± 1.71
Calmness–Nervousness	Control	ll carriers	33.00 ± 1.10	34.73 ± 1.18	34.64 ± 1.42
		ss/sl carriers	33.06 ± 1.16	32.94 ± 1.35	34.38 ± 1.32
	Stress	ll carriers	35.18 ± 1.09	26.82 ± 1.70	32.91 ± 1.46
		ss/sl carriers	32.63 ± 1.05	21.56 ± 1.40	30.50 ± 1.60

TSST, Trier Social Stress Test.

genotype-by-time interaction, GB: $F(2, 50) = 0.55$, $p = .58$; AT: $F(2, 50) = 0.32$, $p = .73$; and genotype-by-stress-by-time interaction, GB: $F(2, 50) = 2.50$, $p = .09$; AT: $F(2, 50) = 0.25$, $p = .78$. Descriptive statistics of the GB, AT, and CN mood scales in each group/condition at each time point are shown in Table 1.

Genotype-dependent memory retrieval

In the presented data, there was no genotype-dependent behavioral alteration. For the data of accuracy, there was no main effect of genotype, $F(1, 25) = 0.43$, $p = .52$; of genotype-by-stress interaction, $F(1, 25) = 1.92$, $p = .18$; of genotype-by-emotion interaction, $F(1, 25) = 0.45$, $p = .51$; or of genotype-by-stress-by-emotion interaction, $F(1, 25) = 0.20$, $p = .66$. For the data of RT for correct response, there was no main effect of genotype, $F(1, 25) = 0.04$, $p = .85$; of genotype-by-stress interaction, $F(1, 25) = 0.14$, $p = .71$; of genotype-by-emotion interaction, $F(1, 25) = 0.15$, $p = .70$; or of genotype-by-stress-by-emotion, $F(1, 25) = 3.58$, $p = .07$. Descriptive statistics of the accuracy and RT of memory retrieval for fearful and neutral faces are displayed in Table 2.

Genotype-dependent brain activity

The whole brain results showed a significant stress-related left hippocampus ([−4 -58 0], k = 228, $z = 4.56$, $p < .01$ FWE cor.) activity increase in the ll carriers, but a decrease in the ss/sl carriers, when retrieving both fearful and neutral faces (see Figure 3A). In the dorsomedial prefrontal cortex (dmPFC, [6 52 22], k = 189, $z = 4.11$, $p < .01$ FWE cor.), we found a decreased deactivation in the ll carriers, but an increased deactivation in the ss/sl carriers during memory retrieval (see Figure 3B).

Genotype-dependent stress-induced behavior/cortisol change that correlated with the hippocampus and dmPFC activity

In order to explore whether the stress related 5-HTTLPR effect in the left hippocampus and dmPFC relate to different behavior or physiological factors, we performed the behavioral/cortisol correlations within the left hippocampus and dmPFC clusters identified above. Since the left hippocampus cluster also extended to areas outside the hippocampus, we restricted the data extraction area only within the hippocampus, using a hippocampus mask derived from

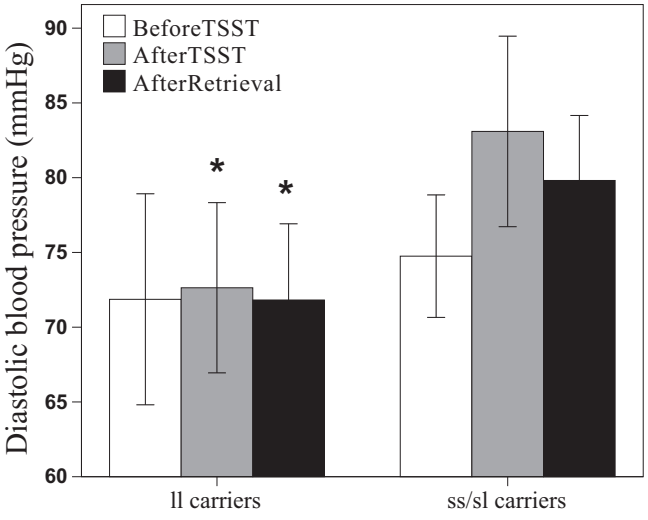

Figure 1. The ss/sl carriers showed significantly higher diastolic blood pressure after the Trier Social Stress Test (TSST) and during the whole retrieval period than the ll carriers in both the stress and control conditions. *.01 < p < .05, ll carriers vs. ss/sl carriers. The error bars represent *SE*.

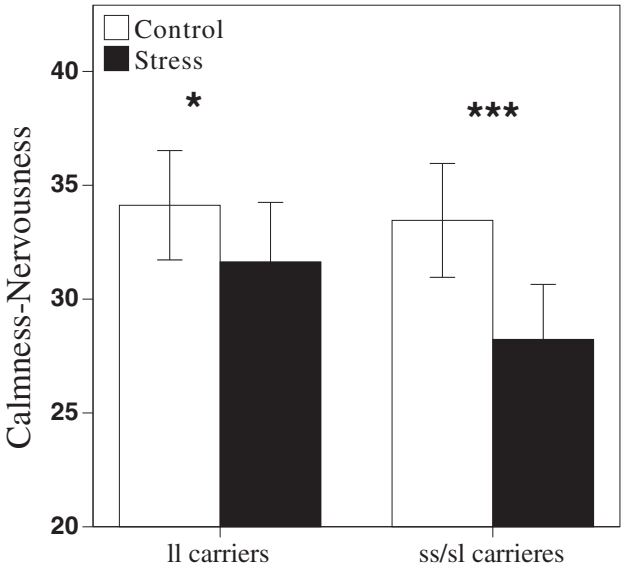

Figure 2. The ss/sl carriers rated themselves to be more nervous than the ll carriers under stress; and both groups showed stronger nervousness under the stress than control condition. *.01 < p < .05; ***p < .001; control vs. stress. The error bars represent *SE*.

the AAL ROI library (Tzourio-Mazoyer et al., 2002). Within the mask, a hippocampus cluster that had the significant peak at [−18, −26, −10], cluster size k = 34, was identified.

Correlation analysis first revealed a significant correlation between the stress-related left hippocampus activity and cortisol $\Delta TSST_{diff}$ in the ll carriers (r = .630, p = .038), but not in the ss/sl carriers (r = −.418, p = .137). A correlation coefficients comparison showed a significant difference between the two groups (z = 2.55, p = .011, two-tailed; see Figure 4A).

Correlation analysis further revealed a significant correlation between the stress-related dmPFC activity and ΔRT in the ll carriers (r = .625, p = .040), but not in the ss/sl carriers (r = −.385, p = .140). A correlation coefficients comparison showed a significant difference between the two groups (z = 2.53, p = .011, two-tailed; see Figure 4B).

Discussion

In the present study, we investigated how 5-HTTLPR influences stress-induced memory retrieval and related neural activity by reanalyzing a relatively small genetic neuroimaging data set. We found that two stress-sensitive brain regions showed distinct genotype-by-stress interaction patterns. During recognition of previously encoded faces after acute psychosocial stress, the ll carriers showed increased left hippocampus activity but the ss/sl carriers showed decreased left hippocampus activity. On the other hand, we found decreased dmPFC deactivation in ll carriers and increased dmPFC deactivation in ss/sl carriers for the same situation. Moreover, genotype-dependent left hippocampus activity correlated with cortisol, while the dmPFC activity correlated with the reaction time.

Our physiological data demonstrated that the ss/sl carriers showed a higher SBP and DBP level than did the ll carriers. As we reviewed in the Introduction, previous studies on the role of 5-HTTLPR genotype in physiological responsiveness to stressful events are inhomogeneous (Taylor, Larson, & Lauby, 2014; Williams et al., 2017). In our study, the baseline DBPs between ss/sl carriers and ll carriers were not significantly different, but after the Control-TSST intervention, ss/sl carriers showed significantly higher DBP than ll carriers. Moreover, the self-rating mood questionnaires showed that both ll carriers and ss/sl carriers were more nervous under the stress condition than under the control condition, while the ss/sl carriers showed stronger stress-related negative mood increases than the ll carriers.

The fMRI results further revealed a 5-HTTLPR-genotype-dependent brain activation pattern in response to acute stress. First, as we expected, the ss/sl carriers showed a distinct stress-induced hippocampal activation pattern when compared with the ll carriers. Specifically, we found decreased hippocampal activation when retrieving facial

Table 2
Stress-related memory retrieval performance in each genotype group

	Condition	Group	Fear (mean ± *SE*)	Neutral (mean ± *SE*)
Accuracy	Control	ll carriers	0.54 ± 0.02	0.55 ± 0.02
		ss/sl carriers	0.53 ± 0.02	0.56 ± 0.03
	Stress	ll carriers	0.52 ± 0.02	0.53 ± 0.02
		ss/sl carriers	0.55 ± 0.02	0.57 ± 0.02
RT (msec)	Control	ll carriers	1605.54 ± 152.19	1486.09 ± 141.48
		ss/sl carriers	1532.47 ± 62.98	1480.53 ± 54.82
	Stress	ll carriers	1513.82 ± 128.04	1571.50 ± 109.94
		ss/sl carriers	155166 ± 77.83	1514.22 ± 83.09

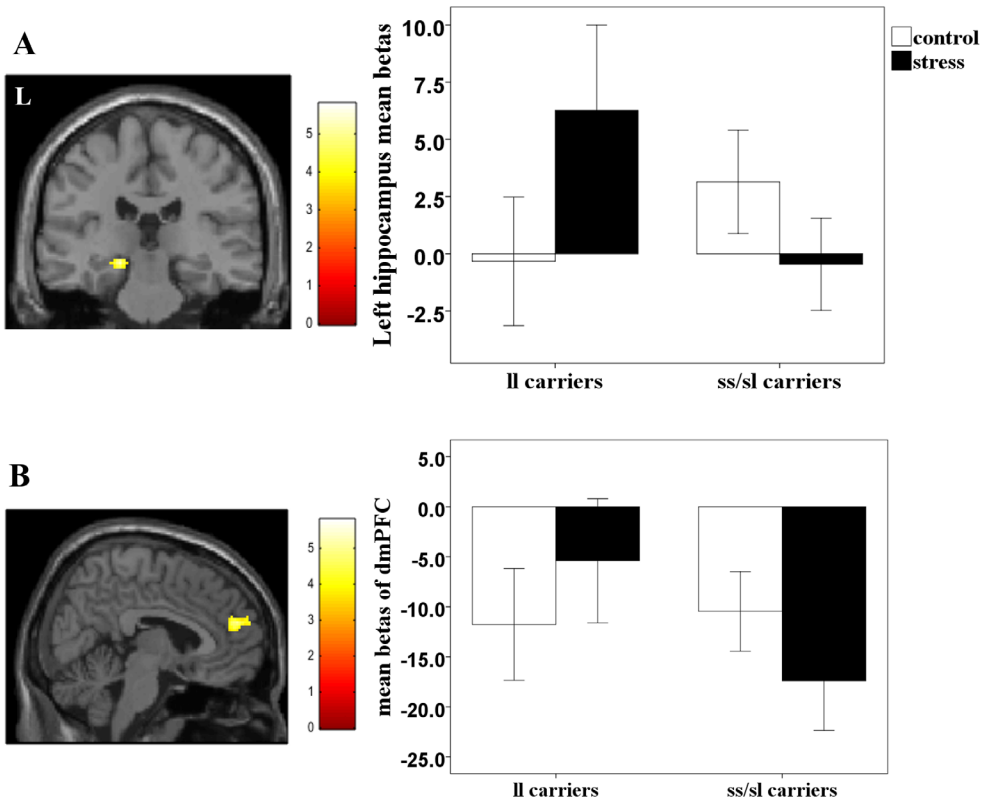

Figure 3. (A) Stress increased the left hippocampus activity in the ll carriers but decreased the left hippocampus activity in the ss/sl carriers. (B) Stress decreased the dorsomedial prefrontal cortex (dmPFC) deactivation in the ll carriers but increased the dmPFC deactivation in the ss/sl carriers. Activations are depicted at $p \leq .05$ using family-wise error corrected at cluster level for the whole brain. The error bars represent *SE*.

memory under stress in the ss/sl carriers, as compared to the increased hippocampal activation in the ll carriers in the same situation. It is well known that the activation in the hippocampus associates with successful memory retrieval (Buckner & Wheeler, 2001; Konishi, Wheeler, Donaldson, & Buckner, 2000; Li et al., 2014; Shannon & Buckner, 2004; Wagner, Shannon, Kahn, & Buckner, 2005) and plays a key role in acute-stress-modulated declarative memory (Henckens, Hermans, Pu, Joels, & Fernandez, 2009; McEwen, 2007; Schwabe & Wolf, 2013) and working memory (Oei, Everaerd, Elzinga, van Well, & Bermond, 2006; Weerda, Muehlhan, Wolf, & Thiel, 2010). In humans, the hippocampus is often involved in the appraisal of a situation by linking the current situation to past experiences (memories) and therefore increases or decreases the stress response accordingly (Schwabe & Wolf, 2013). The hippocampus is involved in a stress-triggered "memory formation mode" that could shape attention and facilitate memory encoding when cognitive processes that are not related to the learning materials (stressor) are suppressed (Herten, Pomrehn, & Wolf, 2017; Schwabe, Joëls, Roozendaal, Wolf, & Oitzl, 2012). However, when retrieving hippocampus-dependent memories, such memory formation mode and partially suppressed cognitive processes might cause retrieval impairment (Buchanan & Tranel, 2008; Kuhlmann, Kirschbaum, &

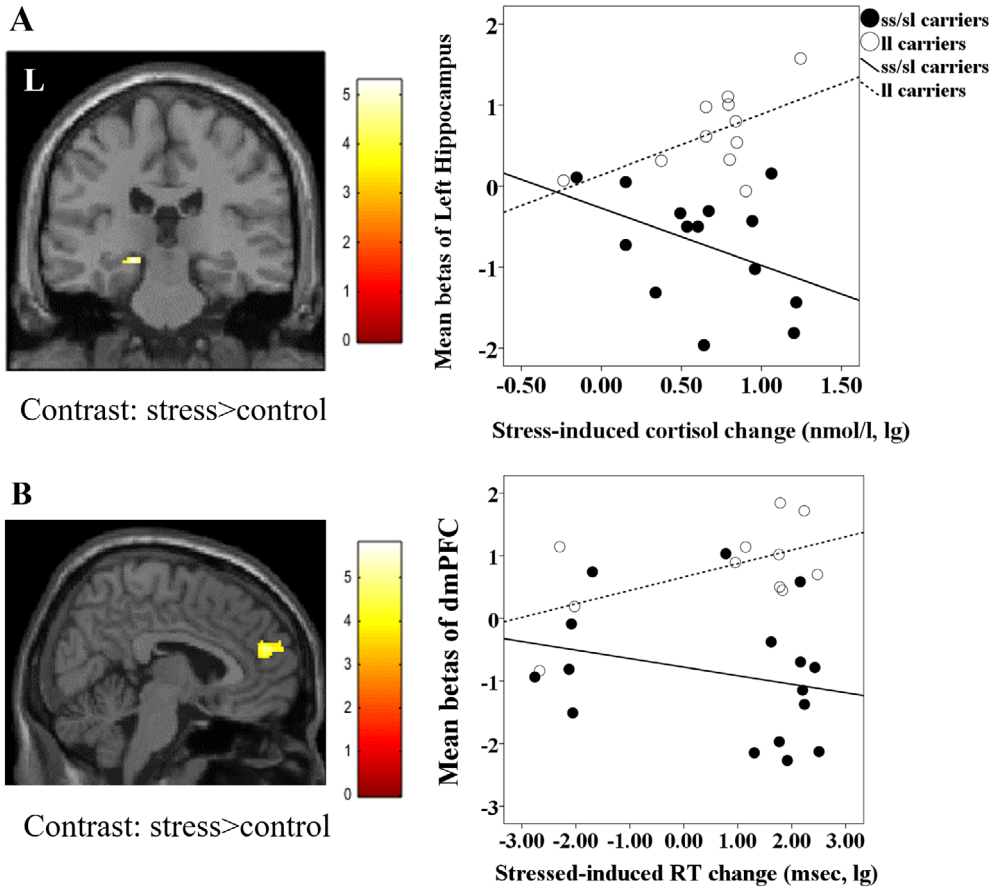

Figure 4. Genotype-dependent left hippocampus and dorsomedial prefrontal cortex (dmPFC) response in relation to (A) stress-induced cortisol change and (B) stress-induced response time (RT) change. The absolute value of cortisol and the RT data were log transformed in order to move the distribution towards a normal distribution. The cortisol data were based on 25 participants.

Wolf, 2005; Kuhlmann, Piel, & Wolf, 2005; Li, Weerda, Guenzel, Wolf, & Thiel, 2013). For instance, cortisone administration reduces hippocampus activation and thereby contributes to memory retrieval deficits (de Quervain et al., 2003; Oei et al., 2007). Moreover, hippocampal engagement during retrieval predicts memory recollection accuracy (Eldridge, Knowlton, Furmanski, Bookheimer, & Engel, 2000; Staresina, Henson, Kriegeskorte, & Alink, 2012; Yonelinas, Otten, Shaw, & Rugg, 2005), and a recent study found that stress disrupted memory recollection by reducing posterior hippocampal engagement (Gagnon, Waskom, Brown, & Wagner, 2018). Memory retrieval impairment under stress occurs rapidly after the cortisol elevation and can persist for at least 90 min (de Quervain, Roozendaal, & McGaugh, 1998; Schwabe & Wolf, 2014; Smeets, Otgaar, Candel, & Wolf, 2008; also reviewed in Wolf, 2017).

A large amount of evidence also supports the interaction between the glucocorticoid system and the serotonin system in learning and memory. Animal studies show that the hippocampus is rich in receptors for the stress hormone corticosterone and that it plays a role in shutting off the HPA axis stress response (Jacobson & Sapolsky, 1991; McEwen, 2007). The serotonergic system is also involved in the hippocampal regulation of the HPA axis activity and increases hippocampal glucocorticoid receptors during development (Andrews et al., 2004; Law et al., 2009; Meaney et al., 2000). O'Hara et al. (2007) found that ss/sl carriers showed higher waking cortisol and have impaired memory recall and a lower hippocampal volume in old age. According to the serotonergic hypothesis of depression, a vulnerable serotonergic system is associated with a heightened sensitivity to stressful events and affective disorders (Fink, 2016). For example, the 5-HT reuptake inhibitor clomipramine increased cortisol and adrenocorticotrophic hormone (Riedel et al., 2002; Stokes, 1995), and higher cortisol levels may also be associated with diminished brain serotonin synthesis (Stokes, 1995). Our results revealed a correlation between acute psychosocial-stress-induced cortisol elevation and hippocampus activity: in ss/sl carriers, participants with higher cortisol elevation after TSST treatment showed less stress-induced hippocampus activation,

whereas in ll carriers, participants with higher cortisol elevation after TSST treatment showed stronger stress-induced hippocampus activation. Even in the absence of behavioral alteration, there was a serotonergic and glucocorticoid interaction on the neural level. An earlier study found that participants carrying both the ll variant of 5-HTTLPR and the TT variant of OXTR (the oxytocin receptor gene) showed lowest fear and sadness personality, as well as the lowest negative emotionality, suggesting that the genotype constellation (5-HTTLPR ll carriers and OXTR TT carriers) might be resilient for anxiety and depression disorders (Montag, Fiebach, Kirsch, & Reuter, 2011). Our findings add new evidence that ss/sl carriers and ll carriers show a differential hippocampal activation pattern with an opposite relation to stress-induced cortisol elevation, which might explain why different genotypes respond to stress/stressful events in distinct ways.

Second, the dmPFC showed a similar genotype-dependent activity pattern to that of the hippocampus. In addition to the hippocampus, the PFC is also highly involved in stress and memory by supporting a top-down cognitive control of memory and evaluation of the surrounding environment (Badre & Wagner, 2007; Lupien & Lepage, 2001), and the execution of decisions (Pabst, Brand, & Wolf, 2013). The human dmPFC is involved in many cognitive processes, such as the appraisal and expression of negative emotion (Etkin, Egner, & Kalisch, 2011), outcome evaluation under uncertainty and threat-related defensive response (Milad et al., 2007), and self-referential knowledge and mentalizing (theory of mind; Amodio & Frith, 2006). An epigenetic study found that 5-HTTLPR (SLC6A4) methylation predicts abnormal resting-state functional connectivity between the amygdala and the mPFC and therefore built a link between the function of the mPFC to the subcortical network and the serotonergic system (Muehlhan, Kirschbaum, Wittchen, & Alexander, 2015). Klumpers et al. (2015) found that 5-HTTLPR ss/sl carriers showed enhanced threat-related dmPFC activation and that such dmPFC activation was correlated with skin conductance and startle reactions. Note that the dmPFC region defined in Klumpers et al.'s study was located in the posterior region of the rostral MFC (prMFC; according to Amodio & Frith, 2006), whereas the dmPFC in the present study was located in the anterior region of the rostral MFC (arMFC). In our study, the dmPFC showed a deactivation rather than an activation as observed in Klumpers et al.'s study. The dmPFC (arMFC) is located in the anterior part of the default-mode network that often shows deactivation during tasks due to a reallocation of processing resources from internal to external sources of information in order to benefit task-relevant brain processing (Andrews-Hanna, Reidler, Sepulcre, Poulin, & Buckner, 2010; Raichle et al., 2001; Raichle & Snyder, 2007). In another study, Ma et al. (2014) found that ss/sl carriers reported greater distress and showed stronger dmPFC activity during negative self-reflection. The dmPFC clusters in Ma et al.'s study included both the prMFC and the arMFC and the ss/sl carriers showed stronger BOLD signal deactivation in both areas during reflection on negative traits of a friend. Moreover, Luo, Yu, and Han (2017) found that the same region (dorsal anterior cingulate cortex [dACC]) showed stronger deactivation in response to death-related words; however, such deactivation did not differ significantly between ss and ll allele carriers. When including cultural traits, such as interdependence, in regression analysis, Luo and colleagues found that in response to mortality threats, dACC/dmPFC activation showed a positive correlation with the interdependence trait in ss carriers but a negative correlation with interdependence trait in the ll carriers. It is possible that the death-related words did not trigger stressful feelings that were as strong as those triggered by the TSST manipulation; therefore, they only observed a genotype-related dmPFC deactivation for individuals with lower interdependence trait. Nevertheless, according to the previous studies, it is possible that decreased dmPFC activity will be an important brain marker for self-related stressful situations and will very likely show a genotype-specific function in response to stressful situations.

Furthermore, the present study revealed that the ss/sl carriers showed stronger dmPFC deactivation under the stress condition as compared with the ll carriers, which might indicate that the ss/sl carriers required more effort to reallocate the cognitive resources under the stress condition. The correlation between RT and dmPFC activation might support the conclusion: in ss/sl carriers, individuals with slower RT after the TSST treatment showed lower stress-induced dmPFC activation (stronger stress-induced dmPFC deactivation); whereas in the ll carriers, individuals with slower RTs after the TSST treatment showed weaker stress-induced dmPFC deactivation. In an earlier study by Ozyurt et al. (2014), the dmPFC deactivation strongly correlated with the RT performance during the recognition phase of facial memory in patients with hypothalamic damage, suggesting an association between the dmPFC

activation and the behavioral performance. The ss/sl carriers might be easily distracted by internal or external events during cognitive tasks with high emotional load (such as the stress situation) and therefore spend longer responding than the ll carriers. Moreover, an abnormal deactivation of the dmPFC is often related to mood disorders, such as MDD (Bermpohl et al., 2009; Zhang et al., 2017), and the 5-HTTLPR s allele is an identified risk factor for depressive trait (Caspi et al., 2003; Gonda et al., 2009) and MDD (Fink, 2016; Haberstick et al., 2016). Our findings might add new neural evidence of relevance for the understanding of gene and environment influences on the development of mental disorders.

Taken together, our results provide more evidence that although both the hippocampus and dmPFC are sensitive to stressful situations during memory retrieval, the two brain regions may play different roles in such stress-related memory processes. For example, during memory retrieval, hippocampus activation is regulated by stress-induced cortisol elevation (de Quervain et al., 1998; Schwabe & Wolf, 2014; Smeets et al., 2008) while dmPFC activation is regulated by stress-induced cognitive processes (Lupien & Lepage, 2001; Pabst et al., 2013) and such cognitive processes might reflect as performance change, such as delayed or shortened RTs. These brain functions might be regulated by the serotonin system; therefore, people with different 5-HTTLPR genotypes show a unique correlation pattern of cortisol/RT and brain activity. We would like to suggest that future memory-retrieval studies could test such serotonin and glucocorticoids system interaction either by adding more genetic neuroimaging studies or using pharmacological methods to reveal the underlying mechanisms of the serotonin and stress interaction in the cognitive process.

The current study is limited by its relatively small sample size and the unbalanced genotype distribution, which was not in Hardy–Weinberg equilibrium (only two heterozygotes). According to a recently published article (Turner, Paul, Miller, & Barbey, 2018), small sample size reduces the replicability of task-based fMRI studies. However, since our main findings about the function of the hippocampus and dmPFC could be supported by several related studies, it is possible that the effect was strong enough to show such trend in a relatively small group. Nevertheless, the correlation between 5-HTTLPR genotypes and stress-related brain regions should be tested in a larger population in the future. Another limitation is the absence of female participants within the sample. Previous studies have suggested the potential role of sex in regulating stress and memory and have shown that male and female participants' memory systems may work differently (even oppositely) under acute stress (Schoofs, Pabst, Brand, & Wolf, 2013; Wolf, Schommer, Hellhammer, McEwen, & Kirschbaum, 2001). Future studies with both male and female participants are needed to better reveal the roles of 5-HTTLPR genotypes in regulating stress-involved memory behaviors and brain activities.

Conclusion

To sum up, the present study identified two stress-sensitive brain regions that are modulated by the 5-HTTLPR genotype during emotional facial memory recognition: the ll carriers showed increased left hippocampus activity and decreased dmPFC deactivation under a stress condition, while the ss/sl carriers showed the opposite pattern. Moreover, the stress-induced hippocampal activation was associated differently with the cortisol elevation after the stress event in the ss/sl carriers and ll carriers while the dmPFC activation was associated oppositely with the behavioral performance in both genotypes. Although the results were based on a relatively small sample size, we think it is still important to show the potential interaction between the serotonergic system and the glucocorticoid system on a neural basis under acute stress. Given the fact that recently a large number of studies has debated the role of 5-HTTLPR genotypes in supporting the gene-by-environment interaction theory in the development of mental disorders, and several meta-analyses have failed to replicate the findings of an interaction of 5-HTTLPR and chronic stress, we think it is important to focus on the effect of acute stress and to discuss the mechanism of gene-by-environment interaction in a controllable situation.

Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this study.

Acknowledgments

Shijia Li is sponsored by Peak Discipline Construction Project of Education at East China Normal University. This

work was supported by National Natural Science Foundation of China (NSFC-31600921), Shanghai Sailing Program (16YF1403200) and China Postdoctoral Science Foundation (2017T100281; 2016M591624), Key Specialist Projects of Shanghai Municipal Commission of Health and Family Planning (ZK2015B01), Programs Foundation of Shanghai Municipal Commission of Health and Family Planning (201540114), and Fundamental Research Funds for the Central Universities (2018ECNU-QKT015). Faces from Essex Face Database were downloaded from the website of Dr. Libor Spacek (http://cswww.essex.ac.uk/mv/allfaces/index.html) and faces from the PICS Database were downloaded from the Psychological Image Collection at Stirling (http://pics.psych.stir.ac.uk/).

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