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Serotonin transporter polymorphism alters citalopram effects on human pain responses to physical pain

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ABSTRACT

Humans exhibit substantial inter-individual differences in pain perception, which contributes to variability in analgesic efficacy. Individual differences in pain sensitivity have been linked with variation in the serotonin transporter gene (5-HTTLPR), and selective serotonin reuptake inhibitors (SSRIs) such as citalopram have been increasingly used as treatments for multiple pain conditions. We combined genotyping, pharmacological challenge, and neuroimaging during painful electrical stimulation to reveal how serotonin genetics and pharmacology interact to influence pain perception and its underlying neurobiological mechanisms. In a double-blind, placebo-controlled procedure, we acutely administrated citalopram (30 mg po) to short/short (s/s) and long/long (l/l) healthy male 5-HTTLPR homozygotes during functional MRI with painful and non-painful electrical stimulation. 5-HTTLPR genotype modulated citalopram effects on pain-related brain responses in the thalamus, cerebellum, anterior insula, midcingulate cortex and inferior frontal cortex. Specifically, citalopram significantly reduced pain-related brain responses in l/l but not in s/s homozygotes. Moreover, the interaction between 5-HTTLPR genotype and pain-related brain activity was a good predictor of the citalopram-induced reductions in pain reports. The genetic modulations of citalopram effects on brain-wide pain processing were paralleled by significant effects on the Neurological Pain Signature, a multivariate brain pattern validated to be sensitive and specific to physical pain. This work provides neurobiological mechanism by which genetic variation shapes brain responses to pain perception and treatment efficacy. These findings have important implications for the types of individuals for whom serotonergic treatments provide effective pain relief, which is critical for advancing personalized pain treatment.

1. Introduction

Pain is an unpleasant sensory and emotional experience and the most common reason people seek medical attention (Melnikova, 2010). However, pain treatment does not achieve analgesia for all individuals: one out of every 1.5 to 8.3 patients achieves effective pain relief (The 2007 Oxford league table of analgesic efficacy), implying potential individual differences in underlying mechanisms and treatment responses. Substantial inter-individual differences pervade all aspects of pain responses, including subjective pain experience (Lanier, 1943; Kim et al., 2004), neural responses to painful stimulation (Coghill et al., 1999; Wager et al., 2013), and responses to pain treatment (Levine et al., 1981; Bruehl et al., 2013). Human studies implicate a significant genetic contribution to inter-individual differences in pain sensitivity (Norbury et al., 2007), chronic pain (Zondervan et al., 2005), and analgesic sensitivity (Mogil et al., 2003; Lötsch et al., 2009), which is paralleled in animal models (Mogil, 1999, 2009; Lötsch et al., 2009). These findings suggest that treatment efficacy depends in part on one's genetic makeup, providing both a challenge and an opportunity for personalized medicine. The high variability in drug efficacy across individuals signals a great need to stratify patients into groups, based on genetic and neurophysiological characteristics, that can help to determine which patient should get which treatment (Dib-Hajj and Waxman, 2014).

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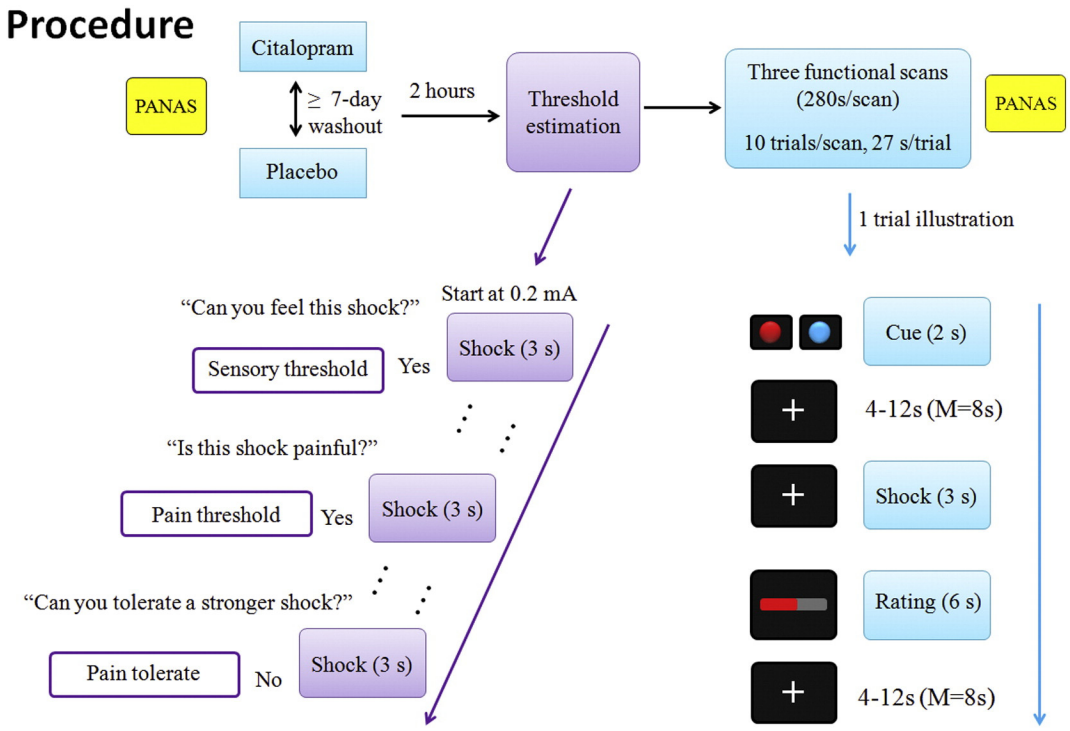

Fig. 1. Illustration of the experimental procedure. Participants' affective states were estimated by the PANAS before citalopram/placebo treatment and after fMRI scanning.

One key determinant of individual differences may lie in genetic variation of the serotonin transporter (5-HTT), a monoamine transporter protein that returns serotonin (5-HT) from the synaptic cleft to the presynaptic neuron. 5-HTT is thought to play a key role in nociceptive processing, as evidenced by 5-HTT knockout rodent studies and human studies (Vogel et al., 2003; Palm et al., 2008; Kupers et al., 2009, 2011; Lunn et al., 2015). For example, 5-HTT knockout mice, which are considered to be a model of lifelong selective serotonin reuptake inhibitor (SSRI) treatment (Bengel et al., 1998; Lesch and Heils, 2000), show reduced sensitivity to thermal pain (Vogel et al., 2003; Palm et al., 2008). A genetic polymorphism in the upstream promoter region of 5-HTT (5-HTTLPR), which has a short (s) and a long (l) variant, has been associated with variation in both clinical pain disorders and experimental pain (Cohen et al., 2002; Marziniak et al., 2005). The variants of the 5-HTTLPR affect the expression, transcriptional activity and function of 5-HTT, with l/l (compared to s/s) homozygotes exhibiting increased 5-HTT expression (Lesch et al., 1996). l/l homozygotes are more sensitive to experimental pain relative to s-allele carriers (Palit et al., 2011; Lindstedt et al., 2011). Moreover, serotonergic drugs, such as SSRIs, have been used for multiple pain conditions (Sindrup et al., 1992; Otto et al., 2008; Lee and Chen, 2010; Lunn et al., 2015), including post-stroke pain, fibromyalgia, and neuropathic pain, though the clinical outcomes of SSRI treatment of neuropathic pain were generally modest (Finnerup et al., 2015).

These findings suggest an important relationship between 5-HTTLPR and pain, but the implications for pain treatment and underlying brain mechanisms remain unclear. Do people of different 5-HTTLPR genotypes respond differently to serotonergic treatments for pain? And, if so, what neural mechanisms underlie this interaction between genotype and treatment? To solve these issues is critical for understanding how individual differences in serotonin genetics modulate the efficacy of SSRI drug, which has important implications for the personalization of pain treatment.

The current work combined genetics, pharmacology, and neuroimaging during painful electrical stimulation to elucidate the neurobiological mechanisms through which 5-HTTLPR affects pain perception and pain treatment. Citalopram is a highly selective SSRI that selectively blocks 5-HTT activity and is associated with antinociceptive effects (Gatch et al., 1998). In a double-blind, placebo-controlled within-subjects crossover design, we acutely administrated 30 mg citalopram, a typical dose used in previous studies (Nandam et al., 2011; Mandrioli et al., 2012; Ma et al., 2015), or placebo to s/s and l/l 5-HTTLPR homozygotes in separate sessions during functional MRI when participants anticipated and received painful and non-painful electric stimulations (Fig. 1). This pharmacogenetic neuroimaging approach allowed us to examine whether and how one's genetic makeup influenced the citalopram efficacy. Numerous neuroimaging studies have consistently shown that brain regions such as the thalamus, insula, midcingulate cortex (MCC), supplemental motor area (SMA) and primary somatosensory cortex encode the intensity of nociceptive stimuli and mediate pain sensation (Peyron et al., 2000; Apkarian et al., 2005; Tracey and Mantyh, 2007; Atlas et al., 2014). In addition, researchers have identified a specific fMRI-based multivariate pattern within and across those regions that discriminates physical pain from social pain, pain anticipation and pain recall with high sensitivity and specificity (Wager et al., 2013, > = 94% in all cases). This pattern, termed the Neurologic Pain Signature (NPS), responds to opiate drug treatment but is not affected by several psychological manipulations in tests to date, including a placebo manipulation (Wager et al., 2013) and a cognitive self-regulation training (Woo et al., 2015). The NPS is thus a useful a priori brain target for studies of individual differences in effects of analgesic drug treatment.

The aforementioned studies motivated several specific, a priori hypotheses. If 5-HTTLPR affects pain treatment, s/s and l/l homozygotes should show differential citalopram-induced changes on pain-related brain responses, and on NPS responses. Specifically, as l/l homozygotes show greater pain sensitivity (Palit et al., 2011; Lindstedt et al., 2011) and increased 5-HTT expression (Lesch et al., 1996), they are expected to exhibit higher pain-related brain activity and NPS responses compared to s/s homozygotes. Moreover, given that l/l relative to s/s homozygotes show stronger SSRI responses in clinical (Hu et al., 2007; Serretti et al., 2007) and laboratory studies (Whale et al., 2000; Ma

et al., 2015), citalopram administration should produce stronger reductions in pain-related brain activity and NPS responses in l/l homozygotes. Finally, stronger brain responses to painful shock—an index of individual differences in hypersensitivity—should predict the magnitude of beneficial citalopram effects on pain. These last two points constitute two effects important for personalized medicine: (a) an interaction between genotype and treatment on pain-related brain responses, and (b) prediction from pain-related brain responses to individual differences in citalopram effects on pain within and across genetic groups, respectively.

2. Materials and methods

2.1. Participants

Fifty-six healthy males, recruited from a pool of 901 university students genotyped for 5-HTTLPR (see below), participated in this study as paid volunteers. Two participants finished the first scanning session, but skipped the second session. Four participants were excluded due to excessive head movement. Thus the final data analyses were performed on 50 participants balanced for 5-HTTLPR genotype: 25 male s/s homozygotes (18–23 years, 19.5 ± 1.7 years) and 25 male l/l homozygotes (18–23 years, 19.1 ± 1.3 years). Age, education, self-esteem and anxiety trait did not differ between s/s and l/l groups (Table S1). All participants were right-handed, and had normal or corrected-to-normal vision. Exclusion criteria included any history of cardiac, hepatic, renal, pulmonary, neurological, psychiatric or gastrointestinal disorders, medication/drug use, and personal or family history of major depression or bipolar affective disorder. We tested only males in this study because we aimed to provide the first test of a complex phenomenon—a Genotype × Treatment interaction—and we wanted to avoid as many potentially confounding variables as possible in this initial test. There are documented sex differences in pain threshold (Chesterton et al., 2003; Kim et al., 2004; Fillingim et al., 2009) and pain-related brain activity (Fillingim et al., 2009; Kano et al., 2013) in the literature. Moreover, pain thresholds vary across the menstrual cycle (Riley et al., 1999; Stening et al., 2007; Fillingim et al., 2009), we aimed to test the interaction (Genotype × Treatment) of primary interest in the study without additional individual variability related to sex and menstrual phase in the initial study. This means that future studies will be required to assess the generalization of these effects to female participants.

The experimental procedures were in line with the standards set by the Declaration of Helsinki and were approved by the Research Ethics Committee of Department of Psychology, Peking University, China. Participants provided their written informed consent after all the experimental procedures had been fully explained, and were acknowledged their right to withdraw at any time during the study. Participants were compensated for their time participated in the study.

2.2. DNA isolation and analysis

We used established polymerase chain reaction (PCR)-based method (Ota et al., 2007) to determine the genotypes of 5-HTTLPR. In a total volume of 50 μl, about 25 ng of genomic DNA was amplified in the presence of 1 × TransStart FastPfu DNA Polymerase (TransGen Biotech) reaction system and oligonucleotide primers (forward 5′-GCATCC CCCATTATCCCCCCCT-3′ and reverse 5′-AGGCTTGGAGGCCGGGATGC-3′) at final concentration of 200 nM. Thermal cycling consisted of 15 min of initial denaturation at 95 °C followed by 35 cycles of 95 °C (20 s), 69 °C (20 s) and 72 °C (15 s) each with a final extension step of 10 min at 72 °C. Subsequently, PCR product was loaded onto a 3% agarose gel (BioWest G-10), to perform electrophoresis to distinguish genotypes of s/s, s/l and l/l. All genotyping was performed in duplicate.

Blood samples of 901 university students (490 males and 411 females, 18–33 years, mean age ± SD = 19.99 ± 2.76 years) were collected for 5-HTTLPR genotyping. Among 901 participants, there were 88 long allele homozygotes (l/l), 194 heterozygotes (l/s), and 619 short allele homozygotes (s/s). The allele frequency is 79% for s-allele and 21% for l-allele, which is similar to those reported in previous studies of Asian populations (Kim et al., 2000; Tsai et al., 2002; Zhang et al., 2009; Zhang et al., 2015).

2.3. Stimulus and procedure

Each participant attended two sessions (at least 7 days apart, ranging 7–29 days, 14.3 ± 6.7 days) and received single doses of citalopram or placebo in each session in a double-blind fully counterbalanced design (Fig. 1). To minimize potential effects of treatment order, we counterbalanced the order of citalopram/placebo treatments within each genotype group and across two groups. Moreover, we estimated the effect of treatment order by running repeated measure analyses of variance (ANOVAs) with treatment order as a between-subject variable and no significant treatment order effect was revealed.

We used a single administration of 30 mg citalopram, a typical dose (20–60 mg, Mandrioli et al., 2012; Ma, 2015) used in previous studies (Nandam et al., 2011; Ma et al., 2015). Before each fMRI session participants completed the Positive and Negative Affect Scale (PANAS, Watson et al., 1988), a 20-item self-report measure of current positive and negative affective states, and then took citalopram or placebo orally. Since pharmacokinetic studies show that citalopram is rapidly absorbed after oral administration, with plasma concentration reaching peak around 2 h for males and a plasma half-life of approximately 35 h, Rocha et al., 2007). Pain threshold assessment and fMRI scanning were commenced after 2 h of treatment administration, including a 1.5 h resting waiting period and a 30-min task familiarization phase. During the waiting period, participants sat on a comfortable couch resting or reading. During the familiarization phase, an experimenter explained the threshold assessment procedure and the task to participants and placed the electrode on the participant's foot for pain threshold assessment. After scanning, participants were asked to (a) rate their fearful, anxious and uncomfortable feelings as related to each of the painful and non-painful stimulations during scanning and (b) to complete the PANAS again.

Three functional runs of 280 s each were obtained from each participant. Each run contained 10 trials (half non-painful and half painful shocks, randomly presented). Each trial started with a 2 s presentation of a cue (a red or blue circle) to indicate painful or non-painful stimulation. The assignment of red vs. blue cue to non-painful vs. painful stimulations was counterbalanced across participants. A fixation-cross of 8 s on average (ranging from 4 to 12 s) was presented after each cue, followed by a 3 s electrical stimulation. Participants were then given 6 s to rate the painfulness of each electrical shock on a visual analog scale (VAS). The VAS was placed horizontally, with "0 = no pain at all" and "10 = worst imaginable pain" presenting at the left and right extremities, respectively. The current rating score was presented above the VAS, and the rating score changed synchronously with the moving cursor on the VAS. The cursor was placed in the middle of the VAS at the onset of painfulness rating. Participants were instructed to move the cursor along the VAS by pressing either of two response keys to indicate their painful feeling induced by each electric shock. After the rating, participants viewed a fixation cross and rested for 6–14 s (average 10 s). Thus, the average total duration of each trial was 27 s; see Fig. 1.

2.4. Pain threshold assessment

The pain task consisted of a pre-scan phase to estimate stimuli thresholds for each individual, a scanning phase during which participants anticipated and experienced electrical shocks in a slow event-related fMRI design, and a post-scan phase to report subjective feelings of electrical shocks. Electrical stimulations were delivered using an fMRI-compatible bipolar concentric surface electrode placed on the dorsum of the left foot of each participant. Each stimulation consisted of a

100-Hz train of 0.5 ms electrical pulses with a duration of 3 s. The current intensity for 'non-painful' and 'painful' shocks was determined on an individual basis. Shocks, starting from 0.2 mA, were applied to participants and were repeated, raising 0.2 mA each time. The current intensity of the shock, to which participants answered "yes" to the question "can you feel this shock?", defined the *sensory threshold*. Experimenter raised the intensity and asked "is this shock painful?" *Pain threshold* was defined as the intensity of the shock to which participants acknowledged pain. *Pain tolerance threshold* was set at the maximum level of current intensity that participants could tolerate by answering "no" to the question "can you tolerate a stronger shock?" (see Table S2 for the stimuli intensity for the sensory and pain tolerance thresholds). The current intensities of sensory threshold and pain tolerance threshold were used as 'non-painful' and 'painful' stimulation during scanning, respectively (see Supplementary Methods for details about adjusted sensory and pain tolerance thresholds).

2.5. Imaging parameters

Functional images were acquired using 3.0-Tesla Siemens-Trio at the Beijing MRI Center for Brain Research. Blood oxygen level dependent (BOLD) gradient echo planar images were obtained using a 12-channel head coil ($64 \times 64 \times 32$ matrix with $3.75 \times 3.75 \times 5.0$ mm spatial resolution, repetition time (TR) = 2000 ms, echo time (TE) = 30 ms, flip angle (FA) = 90°, field of view = 24×24 cm) during the pain task. A high-resolution T1-weighted structural image ($256 \times 256 \times 144$ matrix with a spatial resolution of $1 \times 1 \times 1.33$ mm, TR = 2530 ms, TE = 3.37 ms, inversion time (TI) = 1100 ms, FA = 7°) was subsequently acquired.

2.6. Imaging analysis

Functional images were analyzed using the general linear model (GLM) for event-related designs in SPM8. The functional images were corrected for differences in acquisition time between slices for each whole-brain volume and realigned within and across runs to correct for head movement. The anatomical image was coregistered with the mean realigned image and then normalized to the standard T1 Montreal Neurological Institute (MNI) template. The normalizing parameters were applied to functional images, which were resampled to an isotropic voxel size of $2 \times 2 \times 2$ mm^3 and spatially smoothed using an isotropic Gaussian kernel of 8 mm full-width half-maximum. First-level GLM analyses for each participant included regressors for 'non-painful' cues, 'painful' cues, 'non-painful' shocks and 'painful' shocks, as well as head movement parameters for each run. Events were modeled using a canonical hemodynamic response function. Random-effect analyses were then conducted based on statistical parameter maps from each participant to allow population inference. Significant activations were identified using a threshold of $p < 0.05$ (cluster-level FDR corrected).

The contrasts of 'painful' vs. 'non-painful' cues and 'painful' vs. 'non-painful' shocks identified neural responses during pain anticipation and pain experience, respectively. In two analyses, we subjected contrast images for pain anticipation and experience to a 2×2 factorial analysis. Genotype (s/s vs. l/l) was entered as a between-subjects factor and Treatment (citalopram vs. placebo) was entered as a within-subjects factor. We tested for the effects of Genotype × Treatment interaction in each voxel in the brain. This analysis identified regions in which brain responses differed between citalopram and placebo sessions (Treatment), and whether such citalopram effects differ between s/s and l/l groups (Genotype × Treatment). To further analyze citalopram effects in s/s and l/l homozygotes separately, we conducted whole-brain paired t-tests for Treatment effects on the pain anticipation and pain experience contrasts within each genotype group. These analyses treated participant as a random effect.

In order to visualize the contribution of each condition to the Genotype × Treatment interaction revealed in the whole-brain-analysis, we created spheres with 5-mm radii centered at the peak voxel of the brain regions revealed in the whole-brain Genotype × Treatment interaction. The parameter estimates of signal intensity were then calculated from these regions using MarsBar0.43. Resulting means and standard errors of the mean were plotted. Time courses of 'painful' and 'non-painful' stimulation were also extracted from these regions. This does not provide an independent statistical test on the presence of an interaction, but allows us to visualize the form of the interaction.

In addition to the whole-brain analyses, we applied the NPS to each of the first-level 'painful vs. non-painful' contrast images by calculating the dot product of the image with the NPS pattern, as in previous research (Wager et al., 2013). This provided one 'NPS response' value per condition (Genotype × Treatment, respectively for pain anticipation and pain experience) per participant, or 8 NPS response values per participant, allowing us to test the effects described above on NPS responses.

More specifically, the NPS consists of a specific pattern of activity within and across pain-processing regions, including bilateral dorsal posterior insula, SII, AI, ventro-lateral and medial thalamus, hypothalamus, and dorsal ACC. The signature can be applied prospectively to individual fMRI activation parameter images (i.e., one per participant per condition). The NPS was estimated for each participant in each condition by calculating the dot-product of a vectorized activation image ($\vec{\beta}_{map}$) with the signature pattern $\vec{w}_{map}$, i.e., ($\text{NPS} = \vec{\beta}_{map}^{T}\vec{w}_{map}$), yielding a continuous scalar value. Thus the match between the input image and the pattern weights (calculated as the dot product of the two) provides a single number that reflects the magnitude of the NPS response to that condition. The signature pattern weights were derived from Study 1 in Wager et al. (2013). This neurologic signature was then subjected to 2 (Treatment: citalopram vs. placebo) × 2 (Genotype: s/s vs. l/l) × 2 (Pain: painful vs. non-painful) ANOVAs to assess the effect of treatment and genotype on the representative character of the neural circuit involved in physical pain. The intensities of pain stimulations were included as covariates in the ANOVAs of the NPS to control for potential effects of physical stimulus intensity.

2.7. Scaling of the NPS values for comparability to previous results

The absolute values of the NPS responses are difficult to compare precisely across scanners, in part because the BOLD responses are not quantitative in the sense that values can be compared across scanners; BOLD activity is typically measured and reported in arbitrary units or percent signal change, but calibrating these values so that they are comparable across studies is an ongoing, active field of investigation that requires specialized methods and procedures (e.g., hyper/hypocapnic challenges embedded in the design). Fortunately, the ability to equate BOLD (and thus the NPS) responses across scanners does not impact the ability to make valid comparisons of NPS responses across conditions within a study, including the comparisons across groups (5-HTTLPR genotype: s/s vs. l/l) or conditions assessed within-person (e.g., citalopram vs. placebo, pain vs. non-pain, and their interaction). Thus, the statistical comparisons of the NPS responses reported here did not depend on any scaling factor applied to adjust for overall differences between our scanner and paradigm and those used in previous studies (e.g., Wager et al., 2013), because as with all linear models (applied to BOLD fMRI data or otherwise), the statistical results do not depend on the absolute scale of the responses.

Though we cannot equate the NPS response values to those used in Wager et al. (2013) precisely, we did include an approximate rescaling of the values to make them roughly comparable to the values obtained in Wager et al. (2013). This rescaling was based on four study-level variables that affect the absolute values of the NPS response: field strength, the use of an epoch vs. event-related design, voxel volume, and the

scaling of contrast weights applied to the first-level activation parameter estimates (i.e., beta images). For a more complete discussion of variables relevant for absolute scaling of the activation parameter estimates and our estimated scaling factor, see the Supplementary Materials. Here, we reported NPS responses in units of rescaled contrast estimates, which were 36.5× lower than the raw contrast estimates from the first-level model, though we note that the statistical comparisons we report were identical whether the rescaling is applied or not.

2.8. Regression analyses

We performed moderated regression analyses to examine whether 5-HTTLPR genotype moderated the relationship between the magnitude of brain responses to painful stimulation and treatment efficacy. In the moderated regression model, the independent variable (IV) was *brain sensitivity to pain*, defined as brain responses to painful events under placebo in an individual pain-related brain region—or, in other analyses, the NPS pattern. The dependent variable (DV) was treatment efficacy, defined here as the citalopram effect on pain reports. This effect was calculated as the differential subjective pain reports under placebo minus those under citalopram sessions. Positive values indicated that citalopram decreased pain reports, whereas negative values indicated that citalopram increased pain. The moderator was 5-HTTLPR genotype, coded as a dichotomous dummy variable in which 0 represented s/s homozygotes and 1 represented l/l homozygotes. The interactions between brain sensitivity to pain and genotype were calculated by multiplying the normalized variables together (Aiken and West, 1991). Normalized genotype, IV, and genotype × IV interactions (moderation effects) were sequentially entered into the model. Post-hoc regression analyses were then conducted for each genotype group. This analysis identified whether *brain sensitivity to pain* predicted treatment efficacy, and whether its effects were moderated by genotype. If so, these variables could be used to predict who will respond to citalopram treatment, and thus personalize treatment by prospectively selecting individuals for citalopram treatment who will respond.

3. Results

3.1. Subjective pain reports and mood ratings

The pain reports were defined as the mean trial-by-trial painfulness rating scores of 'painful' stimulation and were then subjected to Treatment (citalopram vs. placebo) × Genotype (s/s vs. l/l) ANOVA. There was no significant Treatment × Genotype interaction ($F(1,48) = 0.21$, $p = 0.65$, Table 1). The mean pain reports tended to be smaller in the citalopram session than in the placebo session, similar to previous report (Gatch et al., 1998), but this effect did not reach significance ($F(1,48) = 0.73$, $p = 0.40$). Citalopram did not significantly affect pain reports in either s/s ($F(1,24) = 0.10$, $p = 0.75$) or l/l ($F(1,24) = 0.69$, $p = 0.42$) groups, motivating additional analyses of individual differences.

The post-scan rating scores of the fearful, anxious and uncomfortable feelings of electric shocks during scanning were subjected to 2 (Pain: 'painful' vs. 'non-painful') × 2 (Treatment: citalopram vs. placebo) × 2 (Genotype: s/s vs. l/l) ANOVAs. There were only significant main effects of Pain on these ratings (Fearful: $F(1,48) = 407.11$, $p < 0.001$; Anxious: $F(1,48) = 378$, $p < 0.001$; Uncomfortable: $F(1,48) = 723.85$, $p < 0.001$, Table S3). No other significant effect was observed (all $p > 0.05$). Self-reported mood changes from pre- to post-scan were subjected to Treatment × Genotype ANOVAs. These analyses did not show any significant effect on either positive (all $p > 0.2$) or negative mood (all $p > 0.3$, Table S4), suggesting a null effect of citalopram treatment on subjects' general mood. Therefore, the observed citalopram effects on pain processing cannot be attributed to citalopram influences on general affective states.

Table 1
Mean (Std. error) trial-by-trial painfulness rating scores to 'painful' and 'non-painful' stimulations.

	s/s homozygotes		l/l homozygotes	
	Placebo	Citalopram	Placebo	Citalopram
Painfulness rating (0 = no pain at all, 10 = worst imaginable pain)				
Pain	8.39 (0.14)	8.25 (0.17)	8.37 (0.16)	8.18 (0.22)
Non-pain	1.07 (0.18)	1.02 (0.12)	0.75 (0.09)	0.90 (0.16)
Pain vs. non-pain	7.32 (0.20)	7.23 (0.17)	7.62 (0.20)	7.28 (0.24)

3.2. Genetic × treatment effects on neural activity during physical pain

To validate the manipulation of electric shocks at a neural level, we first calculated the contrasts of 'painful' vs. 'non-painful' shocks collapsing across s/s and l/l genotypes under placebo. Significant activations were identified using a threshold of $p < 0.05$ (cluster-level FDR corrected). Similar to previous findings (Peyron et al., 2000; Wager et al., 2013), under placebo, painful compared with non-painful shocks increased activity in the typical pain-related circuit, including the bilateral anterior insula (AI), posterior insula (PI), cerebellum, secondary somatosensory area (SII), thalamus (extending to basal ganglia), midbrain, MCC, SMA, and superior parietal cortex (SPC) (Fig. S1B, Fig. S2).

To identify differential citalopram-induced changes on pain-related brain responses between l/l and s/s homozygotes, we conducted a whole-brain factorial analysis to reveal the interaction between Genotype (s/s vs. l/l) and Treatment (citalopram vs. placebo) on pain-related brain activity. This analysis uncovered significant Genotype × Treatment interactions in the bilateral thalamus, cerebellum, right AI, MCC, right lateral inferior and middle frontal cortex (Fig. 2, Table S5). In each of these regions, citalopram reduced neural responses to painful stimulation in l/l homozygotes, but did not significantly influence pain-related brain activity in s/s homozygotes (Fig. 2, Fig. S3 for time courses of BOLD signals of each region). To dissect the meaning of the Genotype × Treatment interactions, we examined the citalopram effects for each genotype group by comparing pain-related activity ('painful' vs. 'non-painful' shocks) in the placebo and citalopram sessions for l/l and s/s groups, respectively. A whole-brain paired t-test showed that, in the l/l groups, citalopram (relative to placebo) significantly decreased the activations in the right AI, thalamus, cerebellum, MCC and SMA (Table S5). A similar analysis comparing placebo and citalopram conditions in s/s homozygotes failed to show any significant effects. Together, these results provided evidence for stronger citalopram effects on pain-related brain activity in l/l than s/s homozygotes.

3.3. Genetic × treatment effects on the Neurologic Pain Signature (NPS)

NPS has been demonstrated to be sensitive and specific to physical pain and analgesic effects (Wager et al., 2013). Thus we further examined the genetic and treatment effects on physical pain-related brain patterns by calculating NPS responses. Here, NPS response values were calculated for each condition within each participant, and subjected to the same analyses as our voxel-wise effects.

First, the NPS responded more strongly to 'painful' than 'non-painful' shocks ('painful': 3.65; 'non-painful': 1.29; $F(1,48) = 421.48$, $p < 0.001$). NPS responses were stronger to physical pain experience ('painful' minus 'non-painful' shocks) than to pain anticipation ('painful' minus 'non-painful' cues; physical pain: 2.36, anticipation: 0.21, $F(1,48) = 400.37$, $p < 0.001$). These findings are consistent with previous finding of stronger responses to physical painful stimulation, and higher sensitivity to physical pain over pain anticipation (Wager et al., 2013). NPS responses to ('painful' vs. 'non-painful' shocks) were then analyzed with Treatment (citalopram vs. placebo) × Genotype (s/s vs. l/l) ANOVAs, which included stimulus intensity as a covariate. During pain

perception, we identified a significant Genotype × Treatment interaction on NPS responses ($F(1,47) = 6.92$ $p = 0.012$, Fig. 3A). Post hoc analyses confirmed that citalopram significantly decreased NPS responses in l/l ($F(1,23) = 6.57$, $p = 0.018$, Fig. 3) but not in s/s homozygotes ($F(1,23) = 1.95$, $p = 0.18$). These results suggested that the Genotype × Treatment interaction was also manifested at the level of a pattern of fMRI activity across multiple pain-related brain regions, consistent with the effects observed within each pain-related brain region.

3.4. Citalopram effect on pain reports: prediction from brain responses to pain reports

Our findings provided evidence for 5-HTTLPR genotype differences in the citalopram effect on pain-related activity. Given that citalopram has been increasingly used in pain treatment, it is important and of clinical interest to evaluate whether brain sensitivity to painful stimulation (measured by the magnitude of activity increases) can predict treatment efficacy, defined here as the citalopram effect on subjective pain

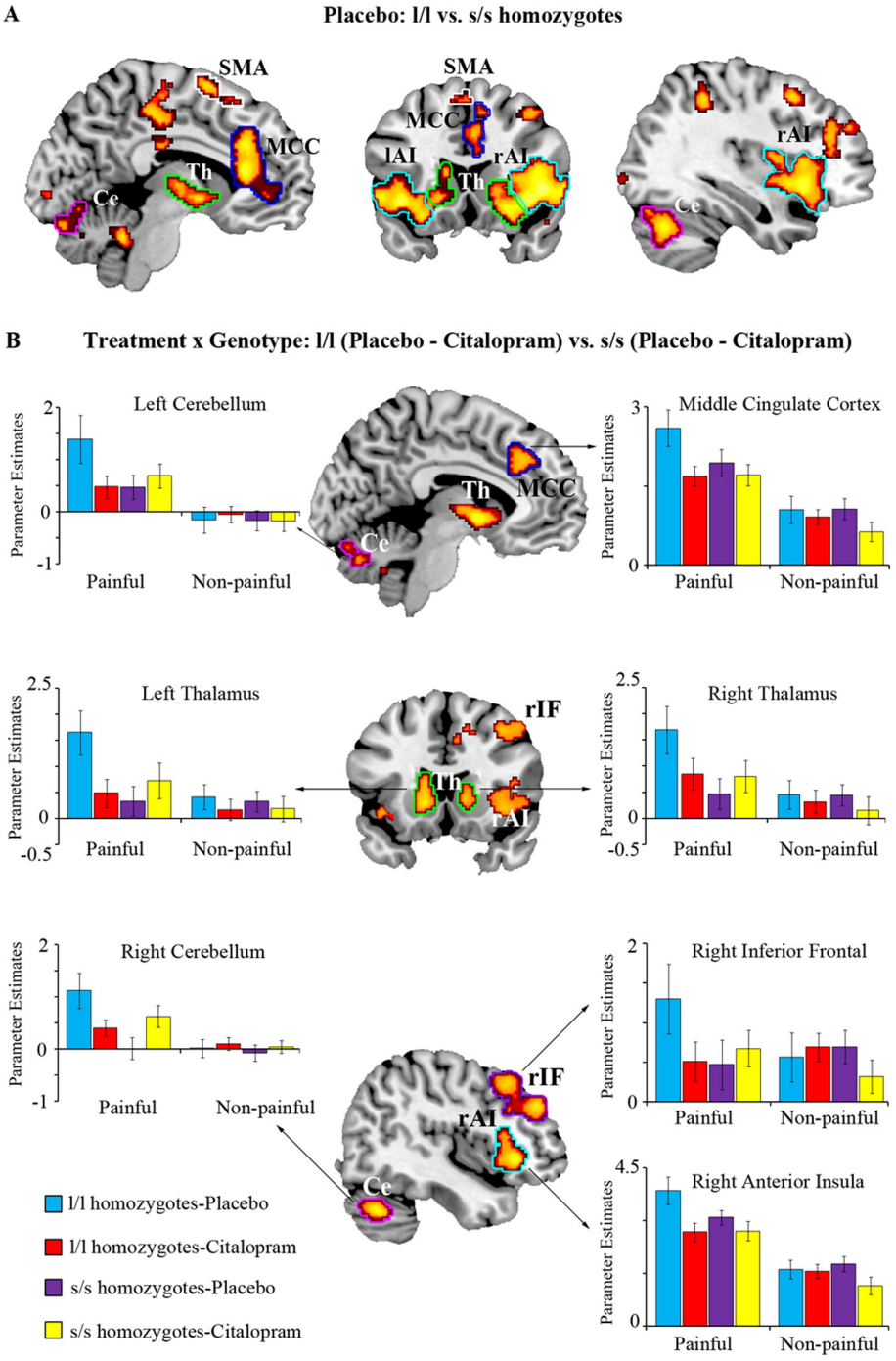

Fig. 2. Genotype × Treatment interaction on physical pain. Significant Genotype × Treatment interaction was observed in the bilateral thalamus, cerebellum, right AI, MCC, right inferior frontal and right lateral middle frontal (at a threshold of $p < 0.05$, cluster-level FDR corrected). The parameter estimates of signal intensity to 'painful' and 'non-painful' shocks were extracted from spheres with 5-mm radii centered at the peak voxel of the brain regions that was revealed in the whole-brain Genotype × Treatment interactions. Resulting means and standard errors of the mean are plotted to illustrate the contribution of each condition to the Genotype × Treatment interaction.

reports. If so, the measurement of baseline pain-related brain responses can be useful in predicting treatment efficacy and guiding treatment decisions. More importantly, we were interested in whether such prediction was moderated by the 5-HTTLPR genotype, which may also be a critical factor. Brain sensitivity to painful stimulation, genotype, and their interaction, were entered as regressors for the regression analyses of the treatment efficacy.

These analyses showed that the relationship between cerebellum/AI sensitivity to painful shocks under placebo and the treatment efficacy was significantly moderated by 5-HTTLPR genotype (right AI: $\beta = 0.47$, $p = 0.001$; left cerebellum: $\beta = 0.55$, $p = 0.002$), suggesting that the interaction between 5-HTTLPR genotype and cerebellum/AI activity was a good predictor for the citalopram treatment efficacy. Post-hoc analyses further revealed that, in l/l homozygotes, citalopram treatment decreased pain reports to a greater degree in those who showed stronger cerebellum/AI activity to painful shocks under placebo (right AI: $\beta = 0.54$, $p = 0.006$, Fig. 4A; left cerebellum: $\beta = 0.44$, $p = 0.027$; Fig. 4B). In contrast, citalopram treatment decreased pain reports to a greater degree in those who showed weaker cerebellum/AI activity to painful shocks under placebo in s/s homozygotes (right AI: $\beta = -0.43$, $p = 0.031$, Fig. 4A; left cerebellum: $\beta = -0.47$, $p = 0.017$; Fig. 4B).

We further found that the relationship between NPS responses to pain under placebo and the citalopram effect on pain reports was also significantly moderated by 5-HTTLPR genotype ($\beta = 0.33$, $p = 0.015$). The NPS response to pain under placebo was a good predictor for citalopram effect on subjective pain reports in l/l ($\beta = 0.54$, $p = 0.007$; Fig. 4C), but not in s/s homozygotes ($\beta = -0.11$, $p = 0.59$; Fig. 4C). Specifically, in l/l homozygotes, the greater NPS responses to painful shocks under placebo, to a greater degree citalopram decreased subjective pain reports. Because the NPS is an a priori marker, unlike other analyses that search for correlations across brain regions, correlations between NPS responses and treatment efficacy constitute unbiased measures of effect size.

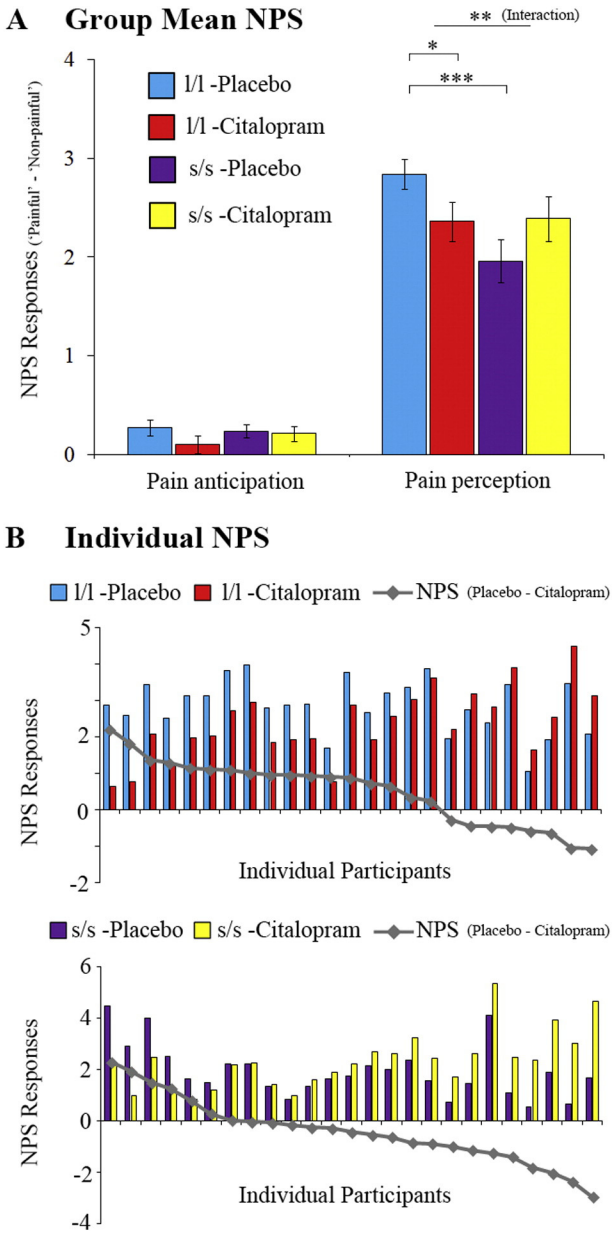

Fig. 3. Genotype × Treatment interaction on Neurologic Pain Signature (NPS) responses. A) Mean NPS responses. B) NPS responses for each participant. NPS responses during pain were greater in l/l than s/s homozygotes under placebo treatment. Citalopram significantly decreased NPS responses in l/l but not in s/s homozygotes.

3.5. Genetic effects on neural activity during physical pain

To test our hypothesis of 5-HTTLPR effects on neural responses to painful stimulation, we conducted a whole-brain two-sample t-test to compare s/s and l/l homozygotes' brain responses to 'painful' vs. 'non-painful' electric shocks under placebo. This analysis revealed stronger activations in the bilateral AI, thalamus, cerebellum, MCC and SMA in l/l relative to s/s homozygotes (Fig. S4, Table S6). In contrast, s/s homozygotes did not show any significantly stronger activation than l/l homozygotes.

3.6. Absent genetic and treatment effects on neural activity during anticipation of pain

Since both pain anticipation and pain experience activated similar brain regions (Ploghaus et al., 1999; Apkarian et al., 2005; Koyama et al., 2005; Bushnell et al., 2013), we investigated whether the genetic and treatment effects on neural responses would be similarly observed during pain perception and anticipation. The contrast of 'painful' vs. 'non-painful' cues revealed significant anticipatory activity in the bilateral AI, cerebellum, SII, thalamus, MCC, SMA and SPC (Fig. S2A). This was consistent with the previous findings that the bilateral AI, cerebellum, SII, thalamus, MCC, SMA, and SPC were commonly activated during pain anticipation and perception (Wager et al., 2004; Peyron et al., 2000; Wager et al., 2013). However, the whole-brain ANOVAs of brain activity involved in pain anticipation did not show any significant effects of Treatment, Genotype or Treatment × Genotype interaction. Similar analyses were conducted on NPS responses during pain perception. ANOVAs of NPS during pain anticipation did not show any significant effects of Treatment, Genotype or their interaction (ps > 0.2, Fig. 3A). The lack of genotype and treatment effects on the neural correlates of pain anticipation in the current study suggested that the Genotype × Treatment interaction might be specific to brain responses during pain perception.

4. Discussion

The current study revealed how serotonergic genetics and pharmacology interact to influence pain-related brain responses, and the underlying neurobiological mechanisms through which 5-HTTLPR modulates the analgesic effect of citalopram. Specifically, we found that acute administration of citalopram significantly reduced pain-related neural activity in the right AI, thalamus, cerebellum, MCC, SMA in l/l homozygotes but no significant citalopram effect was observed in s/s homozygotes, suggesting that the effect of citalopram administration on neural response to physical pain depends on the 5-HTTLPR genotype. The 5-HTTLPR genotype also modulated the relationship between individual's baseline neural response to pain and the

citalopram effects on pain reports. Stronger cerebellum/AI activity to painful shocks (without treatment) predicted greater citalopram-induced pain-report reduction in l/l homozygotes. However, for s/s homozygotes, citalopram treatment decreased pain reports to a greater degree in those who showed weaker cerebellum/AI activity to painful shocks. These genetic modulation effects were paralleled with significant Genotype × Treatment interactions on the NPS — a pattern of activity across multiple brain regions associated with physical pain perception. These results indicate that one's genetic makeup interacts with baseline neural responses to pain to influence the effect of citalopram on pain perception. The current finding has important implications for patient stratification and increasing efficacy of pain treatment.

The current finding may be related to reduced descending inhibition of nociceptive signaling, which is mediated by serotonergic function in a number of animal models of descending pain control (Millan, 2002 for a systematic review). Previous studies of human participants have shown evidence for the involvement of opioidergic and dopaminergic systems in modulations of pain (Bushnell et al., 2013). However, the role for central serotonergic systems has not been well established in spite of the use of SSRIs in treating several forms of pain, such as central post-stroke pain and neuropathic pain (Sindrup et al., 1992; Otto et al., 2008; Lee and Chen, 2010; Lunn et al., 2015). Our fMRI results provide the first pharmacogenetic neuroimaging evidence for the effects of both 5-HTT genotype and SSRIs as well as their interaction on the neural mediators of acute pain, demonstrating a key role of the serotonergic system in acute pain processing and providing a neural basis for further understanding of individual differences in serotonergic treatment efficacy.

The type of interactions observed here — involving a genetic background variable that predicts who will respond to drug treatment and who will not — has significance for advancing personalized medicine. There is a critical need for developing tests that can identify classes of individuals who will and will not respond to treatment, an approach referred as 'stratified medicine' (Kapur et al., 2012), which aims to increase drug efficacy. However, there have been few empirical demonstrations that individual differences in drug efficacy can be predicted (Ma et al., 2015). Regarding clinical treatment of pain, although SSRIs have been used for multiple pain conditions (Sindrup et al., 1992; Otto et al., 2008; Lee and Chen, 2010; Lunn et al., 2015), the efficacy of SSRIs was modest and treatment effects on clinical pain have been overstated in published studies (Finnerup et al., 2015). Our findings identified two strata of individuals (l/l and s/s genotype of 5-HTTLPR) who showed differences in the efficacy of acute SSRI treatment and provide a potential account for the modest SSRI efficacy of clinical pain treatment. Furthermore, our findings indicate that not only one's genetic makeup, but also one's baseline pain responsiveness and their interactions, altered the citalopram effect on pain. We found that the pain-related brain activity predicted individual differences in treatment efficacy within and across 5-HTTLPR genotype groups, respectively. l/l homozygotes responding stronger to painful stimulation and s/s homozygotes responding weaker to painful stimulation are more likely to benefit from citalopram. It has been suggested that variations in pain characteristics between patients influence efficacy of pain treatment and patient stratification on the basis of symptoms would be beneficial on pain treatment (Dib-Hajj and Waxman, 2014). Our results suggest that genetic makeup should be taken into consideration when exploring the relationship between individuals' pain characteristic and treatment effect. Moreover, a simple genetic screen and baseline pain response measure can provide a good estimation of potential citalopram effects on pain reports. Although clinical MRI scans are not yet routinely available for this purpose, findings like these are needed to motivate their use in clinical applications.

It should be noted that the current study did not find significant 5-HTTLPR or citalopram effects on subjective pain reports. This limits the implication of the current findings in clinical populations, but does not

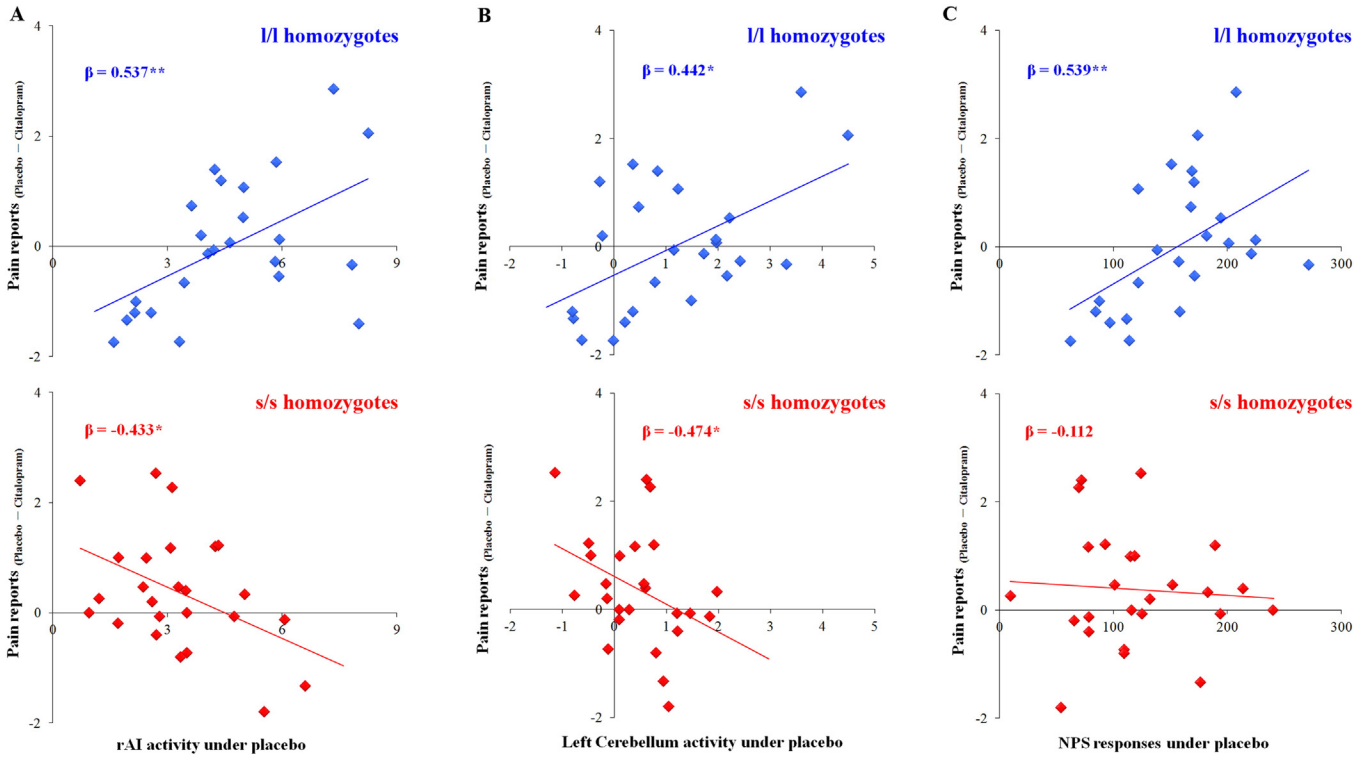

Fig. 4. Relationship between pain-related activity and citalopram effect on subjective painfulness ratings of painful shocks. A/B) Stronger rAI/cerebellum activity to painful shocks predicted greater decreases (for l/l homozygotes) or increases (for s/s homozygotes) in pain reports by citalopram. C) Stronger NPS responses to painful shocks under placebo predicted greater decreases in pain reports by citalopram in l/l but not in s/s homozygotes.

indicate a lack of 5-HTTLPR and citalopram influences on affective states or pain reports in general. It is possible that the effect of single doses of citalopram on subjective feeling was subtle and unable to be picked up with the current sample size. The strong pain stimulation may also lead to limited variability in pain-reports between placebo and citalopram sessions. Although there is no overall genotype/treatment effect on subjective pain reports, AI/cerebellum activity and NPS responses to pain predicted individual differences in the citalopram effect on pain reports. In addition, the pattern of neural responses and subjective reports during pain showed the same direction (i.e., l/l, relative to s/s, homozygotes showed stronger citalopram effect on neural responses and subjective reports during pain).

Pain is a multidimensional experience, involving sensory-discriminative, affective-motivational and cognitive components (Peyron et al., 2000; Bushnell et al., 2013). The contralateral thalamus, SI/SII, and posterior insula underlie pain sensation and encode intensity of pain stimuli (Peyron et al., 2000; Bushnell et al., 1999; Ostrowsky et al., 2002; Apkarian et al., 2005). The medial thalamus, MCC, AI and cerebellum mediate pain-related affective processes of pain, for example, pain-related unpleasantness is associated with activity in the MCC, AI and cerebellum (Rainville et al., 1997; Singer et al., 2004; Craig, 2011; Bushnell et al., 2013) whereas activity increases in the thalamus reflect a general arousal reaction to pain (Peyron et al., 2000). The posterior parietal and prefrontal cortices play key roles in cognitive-evaluative aspects of pain experience (Peyron et al., 2000; Bushnell et al., 1999). To date, little is known about whether the activities in different regions of the pain-related network are similarly modulated by 5-HTT. Our findings revealed that the interaction between serotonergic genotype and drug influenced the thalamus, AI, MCC, cerebellum and SMA activity but did not produce significant effects on the SI, SII, PI and parietal activity to pain, suggesting that 5-HTT plays a more important role in enhancing motor-related and affective (relative to sensory or cognitive) processes during pain experience.

In line with this result, we found that the interaction between 5-HTTLPR genotype and individual's baseline neural response to pain was a good predictor of the potential analgesic effect of citalopram. Stronger baseline cerebellum/AI activity to painful shocks (without treatment) predicted greater citalopram-induced subjective pain-report reduction in l/l homozygotes. This effect was mainly observed in the AI, cerebellum, and MCC (to a less extent, as the interaction between 5-HTTLPR genotype and baseline MCC activity only marginally predicted citalopram effect on pain reports, $\beta = 0.29$, $p = 0.072$). These regions are implicated in the affective and motor-related component of pain (Peyron et al., 2000; Moulton et al., 2010; Bushnell et al., 2013). Expectation of pain activated the medial frontal, AI, MCC and cerebellum (Ploghaus et al., 1999; Chua et al., 1999), and observing another individual in pain activated AI, MCC, cerebellum and brainstem (Singer et al., 2004; Jackson et al., 2005; Xu et al., 2009). Moreover, these regions are particularly important for the subjective experience of pain, encoding subjectively perceived pain intensity (Peyron et al., 2000; Koyama et al., 2005; Baliki et al., 2009) and are sensitive to inter-individual differences (Coghill et al., 2003; Coghill, 2010). Consistently, we showed that interaction between 5-HTTLPR and baseline NPS values predicted citalopram effect on pain report. Given that the NPS pattern mainly reflects sensory and affective aspects of pain (Wager et al., 2013; Woo et al., 2015), these findings together suggest that neural activity in the affective node of the pain network provides a good prediction of individual differences in citalopram effect on subjective pain reports, with the prediction directions depending on the 5-HTTLPR genotype.

The brain activity pattern observed in our work during pain anticipation was different from the previous report of greater neural activity in response to acute stress induced by pain anticipation in s/s than l allele carriers of 5-HTTLPR (Drabant et al., 2012), which was apparently different from our results of absence of genotype differences in brain activity during pain anticipation and the stronger neural activity during experiencing pain in l/l compared to s/s homozygotes. There were several factors that might induce the difference in fMRI results across the studies. First, it has been recognized that, although a similar neural network is shared by anticipation of pain and experience of pain, the neural activity activated during experiencing pain and anticipation of pain can show distinct patterns in the same brain region (e.g., insular cortex and cerebellum, Ploghaus et al., 1999). Similar to Drabant et al. (2012), our previous studies found that s/s compared to l/l homozygotes exhibit greater neural responses to threatening signals such as fearful faces (Ma et al., 2015) and reflection on one's own undesirable personality traits (Ma et al., 2014a). In contrast, l/l homozygotes compared to s-allele carriers are more sensitive to pain stimulation (Palit et al., 2011; Lindstedt et al., 2011) and showed hyperactivity within the pain-related network when experiencing physical pain (current work). Therefore, it is likely that 5-HTTLPR may produce distinct effects on neural activity in response to acute stress induced by anticipation and experience of physical pain. Second, there was a key difference in the design of anticipation of pain between Drabant et al. (2012) and the current work. In our study, an electrical shock followed each cue. This design did not engage uncertainty of pain stimulations and thus might reduce the fear and stress related to pain stimulation. In order to maximally induce stress and prevent habituation, Drabant et al. implemented unpredictability by varying both the number of electric shock trials and temporal unpredictability. This design can increase the stress level during pain anticipation (Monat et al., 1972; Carlsson et al., 2006). Thus the difference in experimental design might influence the 5-HTTLPR effects on brain activity during pain anticipation. Third, Drabant et al. (2012) recruited female participants whereas our work studied male participants. It is well known that the two sexes are different in pain-related behavioral and brain responses (Chesterton et al., 2003; Kim et al., 2004; Fillingim et al., 2009; Fillingim et al., 2009; Kano et al., 2013). Thus the difference in 5-HTTLPR effects on brain activity during pain anticipation across the studies suggests potential interaction between gender and 5-HTTLPR on acute stress. Finally, Drabant et al. (2012) studied Caucasian individuals, whereas our work studied Chinese participants. Thus it is possible that the 5-HTTLPR may produce different effects on neural responses to emotional stimuli in different ethnic samples. Indeed, Lee and Ham (2008) found increased activations in response to angry faces in the bilateral amygdala of the l-allele carriers compared with the s/s homozygotes of 5-HTTLPR in Korean women. This is different from the previous finding of greater amygdala responses to fearful faces in the s-allele carriers than the l/l homozygotes in a Caucasian sample (Hariri et al., 2002). Long et al. (2013) reported that, during a resting state, the l-allele carriers showed significantly reduced functional connectivity between the right amygdala and right frontal pole compared with the s/s homozygotes in a Chinese sample. This pattern is also different from the early finding of significant reduction of the functional connectivity between the amygdala and perigenual ACC in response to fearful faces in the s-allele carriers comparison to l/l homozygotes in a Caucasian sample (Pezawas et al., 2005). The ethnic group differences in modulations of brain activity by the 5-HTTLPR might arise from distinct cultural experiences of the participants as our recent work showed evidence for interactions between 5-HTTLPT and cultural trait (e.g., interdependence) on the brain activity underlying self-reflection (Ma et al., 2014b). The divergent findings across the studies might also reflect a consequence of interactions between 5-HTTLPT and social environments, in which different ethnic groups develop, on brain activity in response to emotions such as physical pain. These should be clarified in future research that employs the same design and compares brain imaging results from participants of the same gender and from the same culture.

SSRIs have been used to treat mood disorders such as depression and anxiety (Ma, 2015) and multiple pain conditions (Sindrup et al., 1992; Otto et al., 2008; Lee and Chen, 2010). What remains unclear is whether the SSRI effects on pain conditions only arise from the effects on negative emotion. A clinical relationship between pain and depression has

long been recognized (Moulton et al., 2010), evidenced by the co-occurrence of pain condition and depression (Katona et al., 2005) and the common brain regions modulated by pain and depression (Chopra and Arora, 2014). However, the experience of pain is different from the fear and anxiety caused by threats of pain (Ploghaus et al., 1999). Future research should further clarify distinct SSRI effects on pain conditions and negative emotion in order to predict SSRI effects in clinical treatment.

In conclusion, this study elucidates the neurobiological mechanisms underlying the serotonergic genetics modulation of SSRI-induced brain changes during physical pain perception. As SSRIs have been increasingly used for multiple pain conditions (Sindrup et al., 1992; Otto et al., 2008; Lee and Chen, 2010; Lunn et al., 2015), the current finding of serotonin genetics and pharmacology interaction has implications for the types of individuals for whom serotonergic treatments may provide effective pain relief. Although our experimental data from healthy volunteers have implications for clinical practice, the generalizability of our findings must be assessed on chronic pain conditions and patient population. Future research should examine the role of 5-HTT and SSRI treatment in patients with various chronic pain conditions, thus to promote personalized pain treatment. Finally, the current finding was observed from a sample of only males, future research should also examine whether the current findings can be generalized to females.

Conflict of interest

The author declares no conflict of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.neuroimage.2016.04.064.

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