Supplementary Material

Serotonin transporter polymorphism alters the citalopram effects on human pain responses

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Supplemental information: 6 tables and 4 figures

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Supplemental Methods

Pain stimulation identification

Because the fMRI environment may influence one's subjectively perceived pain and may change the sensory and pain tolerance thresholds, we used following procedure to identify the stimulus intensity used in the scanner:

For each session (i.e., the citalopram and placebo session, respectively), we first measured sensory threshold and pain tolerance threshold outside the scanner. The out-scanner sensory and pain tolerance thresholds provided baseline thresholds for each participant. The reported 5-HTTLPR x Treatment interaction analysis on sensory and tolerance thresholds was conducted on these intensities.

Subjects were placed in the scanner without changing the position of the electrode. After the localization scanning, we delivered the electric shock as practice trials (starting with the out-scanner sensory thresholds, in increments or decrements of ± 5% of sensory intensity). The current intensity of the shock, to which participants answered "yes" to the question "can you feel this shock?" in the scanner, defined the in-scanner *sensory threshold*. Experimenter then delivered the electric shock with the intensity of the out-scanner pain tolerance threshold (in increments or decrements of ± 10% of the pain tolerance intensity). In-scanner *pain tolerance threshold* was set at the

maximum level of current intensity that subjects could tolerate by answering “no” to the question “can you tolerate a stronger shock?”

We performed statistical analyses on the in-scanner and out-scanner sensory and pain tolerance thresholds. The in-scanner thresholds did not differ significantly from out-scanner thresholds at a group level (ps>0.2, Mean change<10%). Moreover, the main effect of 5-HTTLPR genotype, treatment, or their interaction did not have an effect on the in-scanner vs. out-scanner thresholds (ps>0.4). At the individual level, sensory thresholds were increased in 11 subjects, decreased in 16 subjects, and did not change in 24 subjects. Pain tolerance thresholds were increased in 8 subjects, decreased in 8 subjects, and did not change in 34 subjects. For each participant, the stimulus intensity of the in-scanner pain tolerance threshold (that was adjusted to the scanner environment) was set as the current intensity for ‘painful’ condition during fMRI scanning. The stimulus intensity of the in-scanner sensory threshold was set as the current intensity for ‘non-painful’ condition during fMRI scanning.

Scaling of NPS responses

Below, we briefly review variables important for the overall scaling of the NPS response, and BOLD responses more generally, across studies. While calibration of BOLD responses to reflect absolute quantitative measures is an ongoing, active field of study and it is not possible to precisely equate the scale across studies, it is possible to estimate approximate values for how some acquisition and analysis choices affect the

response scaling, and thereby approximately rescale the values so that they are roughly comparable across studies (though the approximate scaling should not be relied on to make quantitative comparisons across studies).

Acquisition variables affecting BOLD scaling: The amplitude (i.e., percent increase) of stimulus-evoked BOLD responses depends on field strength, TR, TE, acquired voxel size, and flip angle, on the local concentration of water in tissue. For example, Donahue et al. (2009) compared intravascular and tissue BOLD signal in the same individuals at the same spatial resolution (3.5 x 3.5 x 3.5 mm, comparable to our studies) and found that signal amplitude varies log-linearly with TE and somewhat less than linearly with field strength. Thus, there are at least 6 acquisition variables to consider if the absolute BOLD responses are to be compared across studies.

Analysis variables affecting BOLD scaling: The amplitude of estimated BOLD responses depends on the choice of baseline state, stimulus timing that may result in nonlinearity in BOLD responses, physiological noise removal and filtering choices, and the scaling of the hemodynamic response function(s) used, model regressors, and contrast weights. In addition, the choice to analyze percent signal change rather than raw contrast values, the method for converting to percent signal change, and the choice to resample voxels (e.g., resampling to 2 x 2 x 2 mm voxels is the default in SPM) also affect the absolute scaling. Thus, there are at least 9 analysis variables to consider if the absolute BOLD responses are to be compared across studies.

Variables considered in the scaling of NPS responses in the present study:

Contrast scaling: This study included contrasts across 3 runs, with contrast weights of 1 and -1. The contrast values thus reflect the sum across the 3 runs. In Wager et al. 2013, contrast weights were normalized so that positive and negative weights summed to 1 and -1, respectively, across runs. Thus, the contrast scaling results in estimates 3 x larger in the present study, requiring a rescaling factor of 1/3.

Voxel volume: Voxels in this study were resampled to 2 x 2 x 2 mm, in contrast to 3 x 3 x 3 mm resampling used in Wager et al. 2013. This results in values in the present study being 27/8 or 3.38 x larger, requiring a rescaling factor of 1/3.38.

Field strength: This experiment used a 3T scanner, as opposed to the 1.5T scanner used to define the response scaling in Wager et al. 2013. A rescaling factor of approximately 1/2 approximately adjusts for this difference, based on Donahue et al (2009).

Event vs. epoch: This experiment used shocks and was modeled with an impulse (very brief) response convolved with the canonical HRF. Wager et al. 2013 used a sustained, tonic stimulus and an epoch model. Responses to brief events are known to be substantially larger than their equivalent contribution to sustained epochs due to nonlinearity (primarily vascular saturation) in the BOLD response with sustained

stimulation (e.g., Wager et al. 2005; Birn et al., 2001). Therefore, even if the scaling of the hemodynamic response is held constant, activation parameter estimates are likely to be substantially larger for brief events than sustained ones, and they cannot be easily directly compared without an accurate model of the BOLD nonlinearity. Our correction factor is based on Wager et al. 2005, who found that responses to brief events vs. 10 sec sustained trains of stimuli (comparable to shocks vs. sustained heat) resulted in brief events being about 1.8 x larger than sustained events, motivating a rescaling factor in the present study of 1/1.8.

Overall scaling factor: Each of these variables has a multiplicative effect on scaling of the NPS response, and therefore the overall scaling of the contrast estimates is based on the product of the scaling factor from these individual variables, resulting in a scaling factor of 1/36.5 being applied to all NPS response values from the present study. However, we note that the statistical comparisons we report are identical whether the rescaling is applied or not.

Comparable NPS responses to other shock studies both before and after rescaling:

Given the differences in acquisition and analysis choices, the absolute values for the NPS response we observed here appear to be in a reasonable range. We have applied the NPS to shock data from another 3T study (without percent signal change scaling or scaling contrast weights), and have observed values in the range of 70 – 150 (mean 100; Krishnan et al. under review), which is comparable in scale to the data from this study.

After rescaling, the NPS responses from the present study are approximately in the range of the values reported in Wager et al. 2013. The average NPS response in the present study is 3.67 units for pain and 1.29 units for no-pain. In Wager et al. 2013, the pain threshold derived from Study 1 and used across studies was 1.32. Thus, no-pain responses in the present study are typically below threshold, and pain-related responses in the present study are typically well above threshold.

Table S1. Participants' information (Mean (Std. Error))

	s/s homozygotes	l/l homozygotes	Two-sample (t-value)	p
Age	19.5 (0.34)	19.1 (0.25)	0.853	0.398
Self-esteem	28.0 (0.57)	29.0 (0.85)	-1.042	0.303
Anxiety trait	17.4 (1.47)	16.1 (1.24)	0. 686	0.496

Table S2. Mean (Std. Error) intensity of sensory and pain tolerance threshold.

	s/s homozygotes		l/l homozygotes	
	Placebo	Citalopram	Placebo	Citalopram
Sensory threshold (mA)	0.91 (0.08)	1.09 (0.11)	0.89 (0.10)	0.87 (0.07)
Pain tolerance (mA)	2.82 (0.26)	3.21 (0.36)	3.24 (0.42)	3.16 (0.36)

Note: An 2 (Intensity: sensory versus pain tolerance threshold) x 2 (Treatment: citalopram versus placebo) x 2 (Genotype: s/s versus l/l) ANOVA revealed a significant main effect of Intensity ($F(1,48)=114.47$, $p<0.001$), indicating stronger stimulus intensity for 'painful' than 'non-painful' shocks. However, this effect did not differ between the two genotypes and between the treatment conditions (Treatment: $F(1, 48)=0.098$, $p=0.756$; Genotype: $F(1, 48)=0.569$, $p=0.454$; Treatment x Genotype: $F(1, 48)=0.346$, $p=0.559$).

Table S3. Mean (Std. Error) post-scan rating scores to 'painful' and 'non-painful' stimulations, separately on anxiety, fear and un-comfortableness rating scales.

	s/s homozygotes		l/l homozygotes	
	Placebo	Citalopram	Placebo	Citalopram
Anxiety				
Pain	7.24 (0.42)	6.2 (0.55)	6.88 (0.42)	6.4 (0.48)
Non-pain	1.32 (0.33)	0.64 (0.20)	0.60 (0.16)	0.88 (0.18)
Pain vs. Non-pain	5.92 (0.45)	5.56 (0.61)	6.28 (0.44)	5.52 (0.50)
Fear				
Pain	5.84 (0.50)	5.52 (0.52)	6.28 (0.39)	6.08 (0.42)
Non-pain	0.76 (0.21)	0.40 (0.14)	0.52 (0.21)	0.40 (0.15)
Pain vs. Non-pain	5.08 (0.49)	5.12 (0.51)	5.76 (0.43)	5.40 (0.47)
Un-comfortableness				
Pain	7.84 (0.34)	7.24 (0.39)	8.04 (0.29)	8.20 (0.20)
Non-pain	1.40 (0.36)	0.80 (0.27)	1.0 (0.19)	1.16 (0.25)
Pain vs. Non-pain	6.44 (0.47)	6.44 (0.53)	7.04 (0.32)	6.88 (0.32)

Table S4. Affect changes from pre-experiment to post-experiment.

	s/s-placebo		s/s-citalopram		l/l-placebo		l/l-citalopram		Interaction
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	F (p)
Interested	-0.38	0.92	0.08	0.97	-0.44	0.77	-0.46	0.76	1.79 (0.19)
Distressed	0.16	0.90	0.29	0.86	0.08	1.08	0.00	0.98	0.38 (0.54)
Excited	-0.29	1.00	0.13	0.61	0.08	0.57	0.04	0.77	1.86 (0.18)
Upset	-0.28	1.06	-0.38	0.77	-0.48	0.82	0.08	1.06	2.75 (0.10)
Strong	-0.04	1.04	-0.08	0.78	-0.32	0.80	0.04	1.08	1.01 (0.32)
Guilty	0.36	1.04	0.25	0.74	0.24	0.60	0.00	0.40	0.27 (0.61)
Scared	0.46	0.78	0.04	0.86	0.16	0.94	0.31	1.09	2.37 (0.13)
Hostile	-0.04	0.79	0.13	0.68	-0.08	0.70	-0.19	0.80	1.19 (0.28)
Enthusiastic	0.29	0.91	0.08	0.65	-0.04	0.68	-0.19	0.63	0.20 (0.66)
Proud	-0.12	0.97	-0.25	1.11	-0.12	1.13	-0.12	0.86	0.06 (0.80)
Irritable	0.13	0.85	0.08	0.78	-0.20	0.76	0.00	0.89	0.19 (0.66)
Alert	-0.28	0.89	-0.17	0.56	-0.20	0.82	-0.46	0.86	2.17 (0.15)
Ashamed	-0.21	1.02	-0.17	0.48	-0.08	0.81	-0.23	0.51	0.65 (0.43)
Inspired	-0.24	0.60	-0.04	0.62	0.08	0.49	0.00	0.40	1.44 (0.24)
Nervous	0.13	0.80	-0.25	0.44	-0.24	0.66	-0.15	0.78	2.81 (0.10)
Determined	0.12	0.78	0.00	0.59	-0.16	0.47	-0.19	0.75	0.25 (0.62)
Attentive	-0.13	0.68	-0.17	0.64	-0.12	0.73	-0.23	0.59	0.21 (0.65)
Jittery	0.20	0.87	0.00	0.66	0.04	0.84	0.08	0.98	0.39 (0.53)
Active	-0.17	0.92	0.04	0.91	-0.12	0.88	-0.08	0.84	0.20 (0.66)
Afraid	0.00	1.12	-0.13	0.99	-0.28	0.98	-0.04	0.96	0.70 (0.41)
Positive-All	-0.13	0.46	-0.04	0.27	-0.14	0.31	-0.17	0.26	0.90 (0.35)
Negative-All	0.10	0.35	-0.01	0.23	-0.08	0.29	-0.02	0.34	2.65 (0.11)

Positive-All: mean rating scores of all the positive affect items
Negative-All: mean rating scores of all the negative affect items

Note: Affect changes measured using the PANAS from baseline (prior to citalopram/placebo treatments) to post-scan were subjected to 2 (Treatment: citalopram vs. placebo) x 2 (Genotype: s/s vs. l/l) ANOVAs, which did not show any significant effect on either positive affect (Treatment: $F(1,48)=0.324$, $p=0.572$; Genotype: $F(1,48)=2.083$, $p=0.303$; Treatment x Genotype: $F(1,48)=1.295$, $p=0.261$) or negative affect (Treatment: $F(1,48)=0.480$, $p=0.492$; Genotype: $F(1,48)=0.751$, $p=0.391$; Treatment x Genotype: $F(1,48)=0.497$, $p=0.484$).

Table S5. Placebo vs. Citalopram effects on the brain activity in responses to pain experience.

	x/y/z (MNI)	T-value	k (cluster size)
Placebo vs. Citalopram effect in l/l vs. s/s genotype			
Thalamus (R)	12/-6/6	5.43	766
Thalamus (L)	-12/10/0	5.14	885
Cerebellum (R)	36/-66/-34	4.82	2362
Cerebellum (L)	-18/-70/-34	4.45	
Anterior insula (R)	42/26/-2	4.37	715
Middle frontal (R)	46/22/46	4.20	822
Inferior frontal (R)	42/40/32	3.91	
Middle cingulated (R)	18/30/38	3.89	522
Medial frontal (L)	-10/30/38	3.79	
Placebo vs. Citalopram effect in l/l genotype			
Thalamus (L)	-16/8/16	5.70	2275
Thalamus (R)	14/-8/8	5.20	1561
Anterior insula (R)	36/18/4	3.98	
Cerebellum (R)	36/-58/-44	4.75	2143
Cerebellum (L)	-20/-72/-34	4.08	
Middle cingulate	18/32/40	4.29	663
Supplementary motor area	-10/22/60	4.29	341
Placebo vs. Citalopram effect in s/s genotype			
No significant regions			

Note: In the “Placebo vs. Citalopram effect in l/l vs. s/s genotype” contrast, the third cluster with 2362 voxels covers the left and right cerebellum. The fifth cluster covers both the middle and inferior frontal cortex with a cluster size of 822. The last cluster covers both the middle cingulated and medial frontal cortex with a cluster size of 522. In the “Placebo vs. Citalopram effect in l/l genotype” contrast, the second cluster with 1561 voxels covers the right thalamus and right anterior insula. The third cluster covers both the left and right cerebellum with a cluster size of 2143.

Table S6. Genotype differences in the pain-related brain activity under placebo (l/l vs. s/s genotype).

	x/y/z (MNI)	T-value	k (cluster size)
Anterior insula (L)	-42/12/-6	5.13	868
Anterior insula (R)	46/18/2	4.47	1057
Thalamus (L)	-10/2/-4	4.02	358
Thalamus (R)	14/-8/8	4.73	201
Cerebellum (L)	-28/-68/-30	4.21	330
Cerebellum (R)	34/-66/-32	3.84	199
Middle cingulate	4/38/14	4.30	980
Supplementary motor area	10/30/54	4.58	533

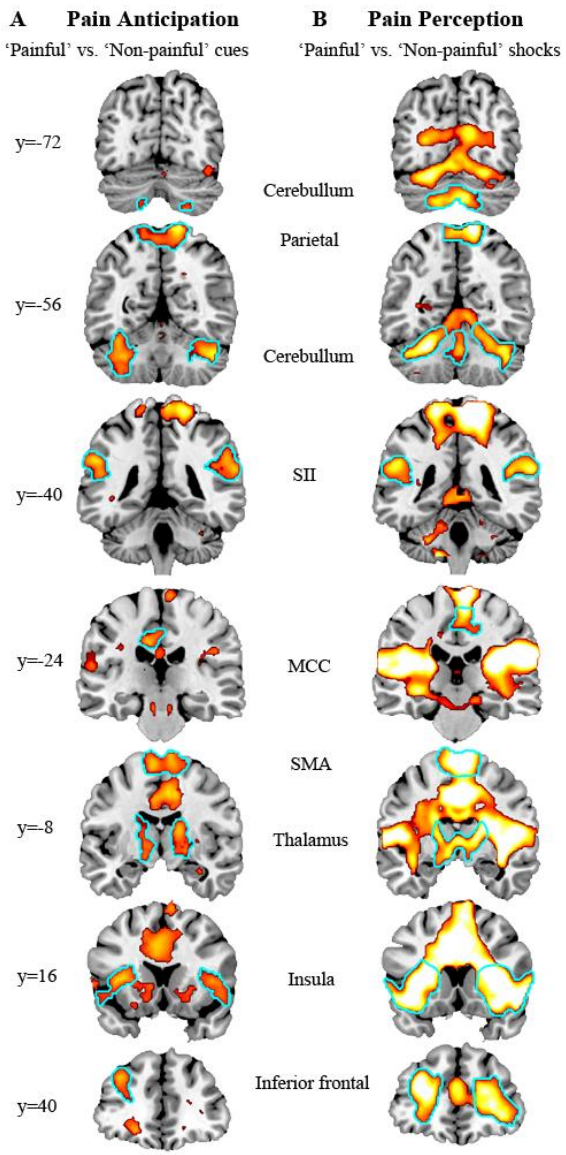

Figure S1. Neural responses to pain anticipation and pain experience collapsing across s/s and l/l genotypes in the placebo session.
A) The contrast of 'painful' vs. 'non-painful' cues leads to significant activation in the bilateral AI (left: -38/18/6; right: 44/24/2), cerebellum (left: -38/-60/-26; right: 46/-60/-30), SII (left: -56/-36/22; right: 60/-40/22), MCC (0/18/34), thalamus (left: -12/10/-8; right: 12/8/-4), and superior parietal cortex (14/-50/72).
B) The neural correlates of pain perception were identified in the bilateral AI (left: -38/10/-8; right: 38/10/-2), PI (left: -34/-22/16; right: 36/-20/16), cerebellum (left: -34/-58/-30; right: 24/-64/-20), SII (left: -56/-30/18; right: 54/-34/26), MCC (-2/14/40), thalamus (left: -20/-14/18; right: 16/-8/10), midbrain (left: -8/-20/-20; right: 6/-4/-2), SMA (6/-6/72), and superior parietal cortex (left: -14/-46/70; right: 18/-44/68). Green circle illustrated the labeled brain regions.

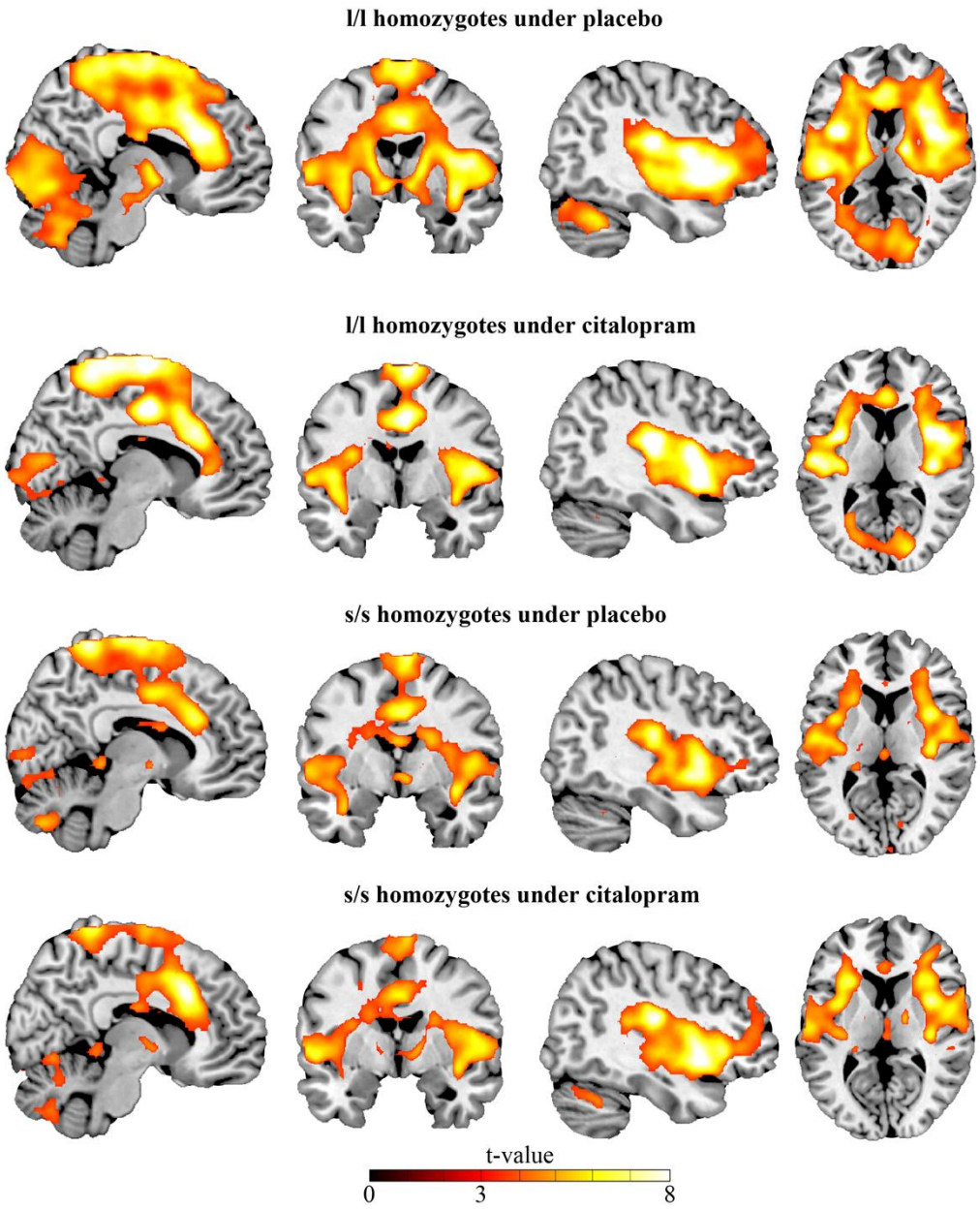

Figure S2. Neural responses to pain experience (i.e., contrast of 'painful' versus 'non-painful' shocks) for l/l and s/s homozygotes under placebo and citalopram, respectively.

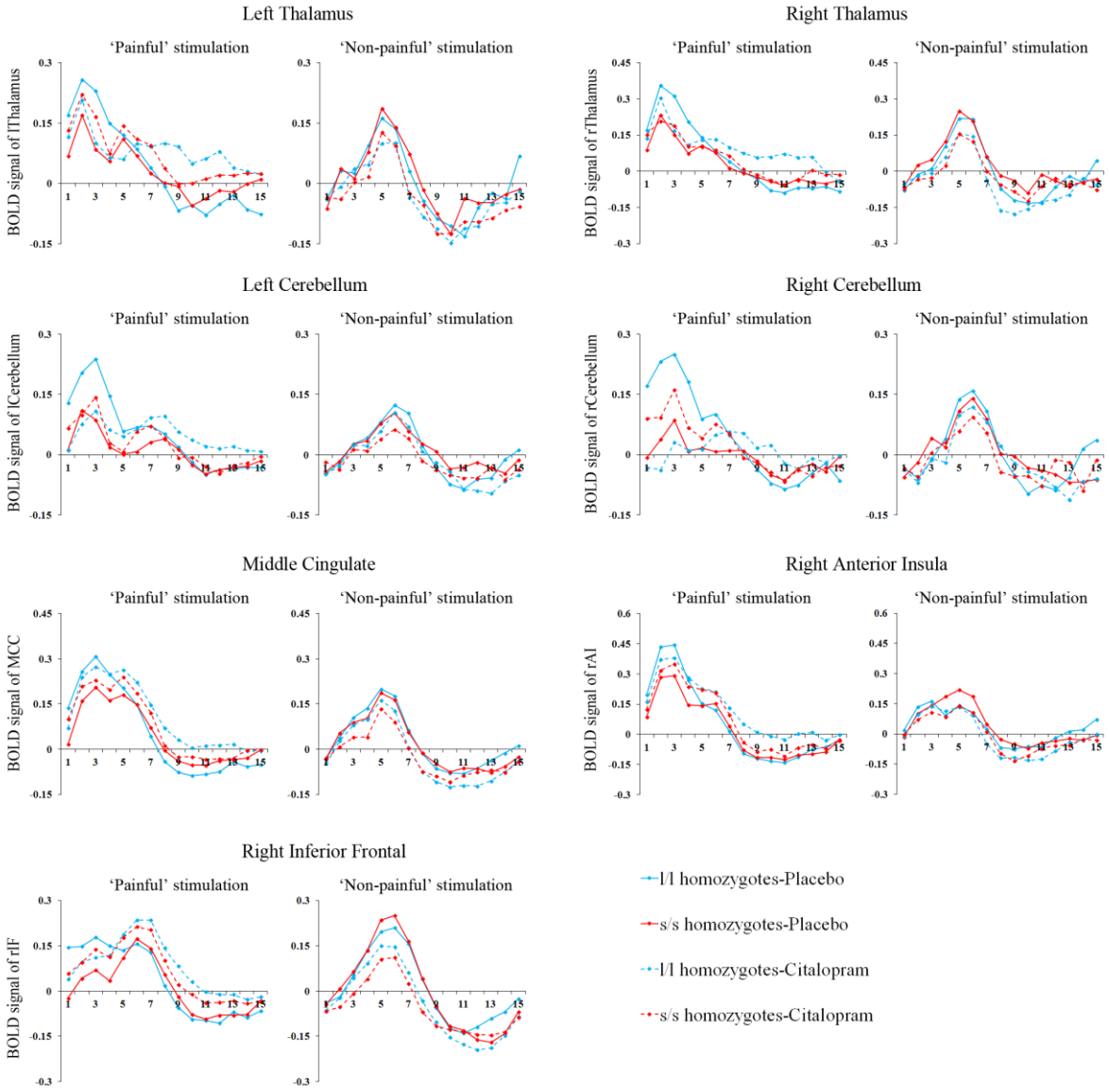

Figure S3. BOLD signals in response to of 'painful' and 'non-painful' stimulation in pain-related brain regions that showed significant Genotype x Treatment interaction in the whole-brain analysis. Time-point 1 represents the onset of 'painful' or 'non-painful' electric shock.

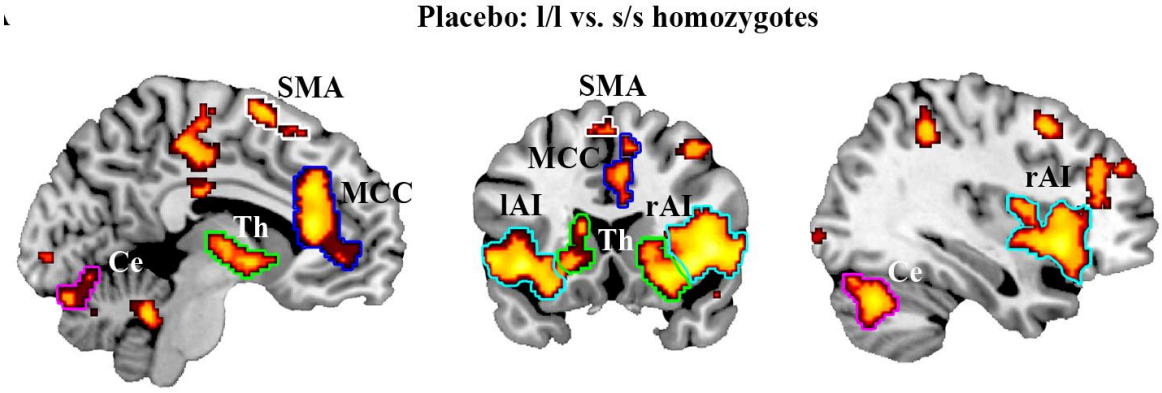

Figure S4. Relative to s/s, l/l homozygotes showed stronger activation in the bilateral AI, thalamus, cerebellum, MCC and SMA under placebo.