SUPPORTING INFORMATION

Effects of chondroitin sulfate on brain response to painful stimulation in knee osteoarthritis patients.
A randomized, double-blind, placebo-controlled fMRI study

S1 Text. Methods

Eligibility and exclusion criteria

Eligibility criteria. A subject was eligible for inclusion when all of the following criteria applied: (i) male or female with age between 40 and 75 years; (ii) diagnosis of primary osteoarthritis and suitability for the study as determined by the responsible rheumatologist, based on a comprehensive medical evaluation; (iii) radiological and clinical osteoarthritis based on the American College of Rheumatology (ACR) criteria;[1] (iv) osteoarthritis radiological grade II or III;[2] (v) stable knee symptoms for at least 1 month prior to screening; and (v) a minimum knee pain severity of 5 points on the 11-point numerical rating scales (NRS) at baseline.

Exclusion criteria: (i) clinical evidence or history of drug/alcohol addiction; (ii) previous adverse effect to CS; (iii) relevant, non-controlled medical or psychiatric disease; (iv) formal MRI contraindication; (v) severe pain in other joints; (vi) inflammatory or systemic diseases with potential repercussion on joints; (vii) secondary causes of arthritis of the knee; (viii) the use of any analgesic, cyclooxygenase-2 (COX-2) inhibitor or nonsteroidal anti-inflammatory drugs (NSAIDs) within seven days prior to inclusion or during the study; (ix) systemic use or local corticosteroid injection three months prior study and during the study; (x) use of CS, diacereine, glucosamine or other symptomatic slow acting drugs for osteoarthritis three months prior the study; (xi) hyaluronan injections into the index knee within the previous six months prior to the study; (xii) subjects smoking over 20 cigarettes a day; (xv) initiation or change of a physiotherapy program in the 2 weeks prior to screening or during the study period; and (xvi) females of childbearing potential.

Sample size assumptions

As there is no consensus as to sample size estimation for fMRI studies, in the current study we used previous data on the same topic by our research team (see Suppl. material for sample size assumptions). After assessing fMRI brain response to pressure painful stimulation in 22 fibromyalgia patients compared with 22 matched control subjects (non-published data), we found a mean between-group difference in fMRI signal in the anterior cingulate cortex of 1.75 units (fMRI beta values) and a global standard deviation of 2.10 units. If the true difference between conditions is 1.75 units, we needed to assess 24 valid subjects in the experimental group and 24 subjects in the placebo group to be able to reject the null hypothesis with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05. Assuming 20% of withdrawals during the study four-month period, a minimum of 60 patients should be randomized. Similar sample size estimates were obtained using data from a study with osteoarthritis patients.[3]

Randomization procedure

Subjects were assigned a randomization number following the chronological order by which they were visited. Post-randomization dropouts were not substituted (randomization numbers were not re-assigned). The randomization list was generated using the: procPlan of SAS System (version 9.2, SAS Institute Inc., Cary, NC, USA http://www.sas.com/) software in blocks that were multiples of two subjects and following a 1:1 pattern. Independent pharmacists dispensed to the investigator the study medication according to the randomization list, while the investigators remained blind to the treatment assignment.

REFERENCES

1 Altman RD. Criteria for classification of clinical osteoarthritis. *J Rheumatol Suppl* 1991; 27:10-2

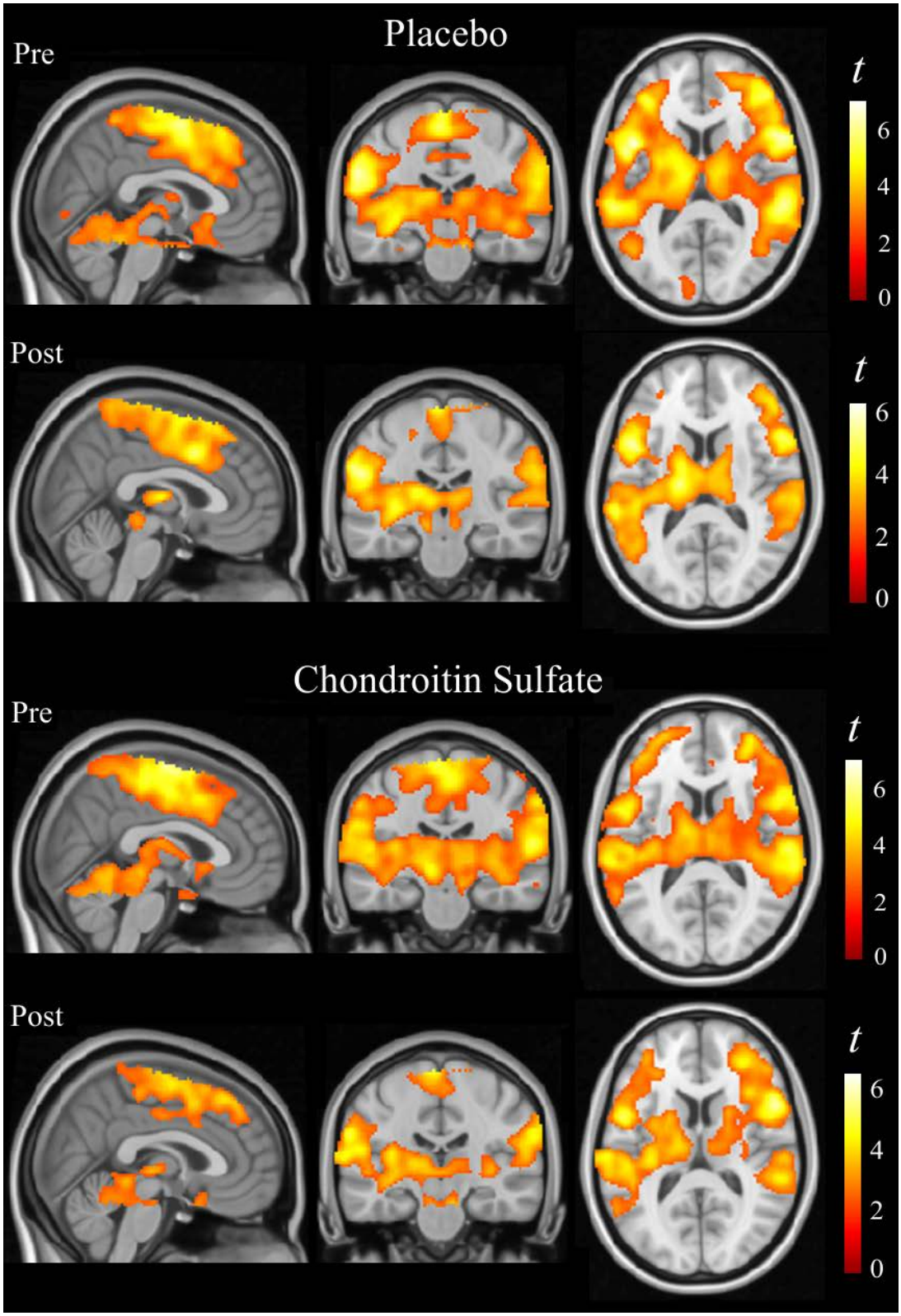
2 Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. *Ann Rheum Dis* 1957;16: 494–502

3 Giménez M, Pujol J, Ali Z, et al. Naproxen effects on brain response to painful pressure stimulation in patients with knee osteoarthritis: a double-blind, randomized, placebo-controlled, single-dose study. J Rheumatol. 2014 Nov;41(11):2240-8.

SUPPLEMENTARY FIGURES

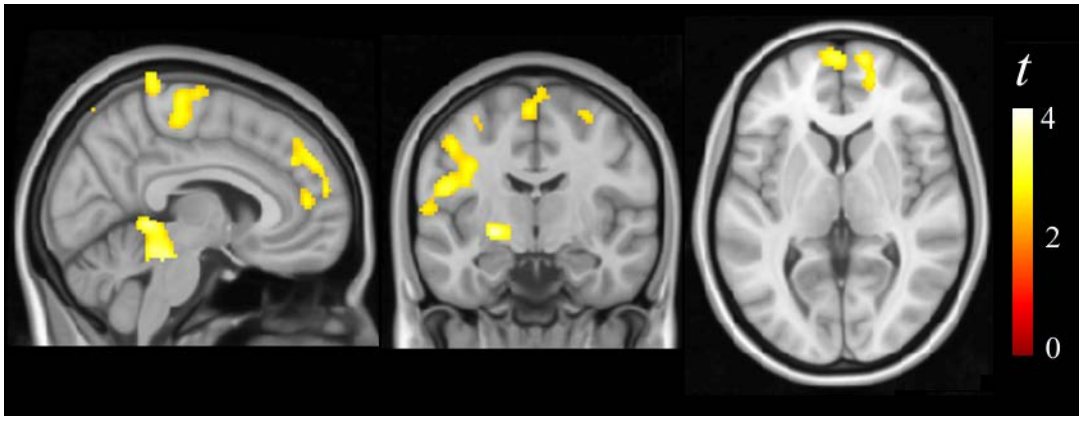
S1 Figure. Supplementary Figure 1

Brain activation (one-sample t-tests) obtained during Knee Interline Pressure Test for both groups and conditions. The test generated a robust activation of the entire pain-related brain system. Data are displayed showing $P_{\text{False Discovery Rate-corrected}} < 0.05$. Right in axial and coronal views corresponds to the right hemisphere.



S2 Figure. Supplementary Figure 2

Between-group differences in baseline brain activation during Patella Pressure Test. Right in axial and coronal views corresponds to the right hemisphere.



SUPPLEMENTARY TABLES

S1 Table. Supplementary Table 1

T1. Knee Interline Pressure Test fMRI results.

	Peak activation							
	Placebo				Chondroitin Sulfate			
Basal fMRI session	**x**	**y**	**z**	**t**	**x**	**y**	**z**	**t**
Sensorimotor cortex- SMA	-2	-14	68	5.8	0	-12	68	6,3
SII- Posterior insula	62	-32	18	7.0	64	-36	26	7.1
Anterior insula-Basal ganglia	40	20	-8	6.0	38	20	-14	6.1
Thalamus	-10	-12	12	5.1	-10	-10	12	4.4
Anterior cingulate-Medial frontal	8	10	40	5.4	10	16	40	5.3
PAG region	-4	-26	-2	3.3	6	-28	-6	4.1
Prefrontal cortex	42	42	16	6.1	46	34	-8	5.1
Final fMRI session								
Sensorimotor cortex-SMA	-2	-14	68	4.3	-6	-10	68	5.2
SII- Posterior insula	60	-34	22	6.3	64	-38	28	6.6
Anterior insula-Basal ganglia	38	24	-4	3.5	38	20	-12	5.8
Thalamus	-16	-16	12	5.2	-14	-16	8	4.6
Anterior cingulate-Medial frontal	2	10	40	5.0	8	12	44	4.2
PAG region	-6	-28	-6	3.6	-12	-34	-12	4.3
Prefrontal cortex	40	46	10	3.8	32	44	12	4.5

All activations are $P_{\text{False Discovery Rate- corrected}} < 0.05$. X, Y, Z, Montreal Neurological Institute- MNI coordinates. SMA, supplementary motor area; SII, second somatosensory cortex. PAG, periaqueductal gray.

S2 Table. Supplementary Table 2

T2. Patella Pressure Test fMRI results

	Peak activation							
	Placebo				Chondroitin Sulfate			
Basal fMRI session	**x**	**y**	**z**	**t**	**x**	**y**	**z**	**t**
Sensorimotor cortex- SMA	0	-14	64	6.2	-2	-12	64	7.0
SII- Posterior insula	54	-32	20	9.5	52	-32	18	6.9
Anterior insula-Basal ganglia	38	20	-4	5.4	40	22	-8	5.9
Thalamus	14	-14	6	3.3	-12	-18	4	5.0
Anterior cingulate-Medial frontal	10	8	42	4.4	10	12	34	5.0
PAG region	-4	-28	-5	2.5	4	-38	-16	4.4
Prefrontal cortex	40	42	12	3.9	38	52	16	5.2
Final fMRI session								
Sensorimotor cortex-SMA	-2	-10	64	5.1	-4	-12	68	4.8
SII- Posterior insula	54	-32	20	7.5	54	-32	20	5.7
Anterior insula-Basal ganglia	42	24	-8	3.2	38	26	-8	5.1
Thalamus	14	-14	6	3.2	-10	-16	4	5.1
Anterior cingulate-Medial frontal	-6	-6	44	4.2	8	14	34	3.1
PAG region	-	-	-	-	8	-28	-14	2.8
Prefrontal cortex	42	38	20	4.2	40	50	8	4.4
Basal>Final within-Group	**Peak differences**							
	x	**y**	**z**	**t**	**x**	**y**	**z**	**t**
Sensorimotor cortex (278 voxels)	-	-	-	-	0	-6	70	2.8
Group by Session Interaction	**Peak interaction**							
(CS Basal>Post)>(Placebo Basal>Post)	x	y	z	t	cluster size			
PAG region	0	-40	-18	2.9	238			

All activations are corrected $P_{\text{False Discovery Rate}} < 0.05$. X, Y, Z, Montreal Neurological Institute-MNI coordinates. SMA, supplementary motor area; SII, second somatosensory cortex. PAG, periaqueductal gray. CS, Chondroitin sulfate.

S3 Table. Supplementary Table 3

T3. Patella Pressure Test fMRI results. Baseline between-group differences.

	Peak differences				
Chondroitin sulfate > Placebo	x	y	z	t	cluster size
Prefrontal cortex	-20	52	18	3.3	1021
Brainstem/Cerebellum	-6	-36	-20	3.9	1753
Sensorimotor cortices- SMA	-50	-18	46	3.5	1272
Basal Ganglia	-24	-14	-6	4.1	295

All differences are $P_{\text{False Discovery Rate- corrected}} < 0.05$. X, Y, Z, Montreal Neurological Institute- MNI coordinates. SMA, supplementary motor area