Effects of chondroitin sulfate on brain response to painful stimulation in knee osteoarthritis patients.

A randomized, double-blind, placebo-controlled fMRI study

Efectos del condroitín sulfato sobre la respuesta cerebral a la estimulación dolorosa en pacientes con artrosis de rodilla.

Un estudio de RMf aleatorizado, doble ciego y controlado con placebo

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ABSTRACT

Introduction. Knee osteoarthritis is causing pain and functional disability. One of the inherent problems with efficacy assessment of pain medication was the lack of objective pain measurements, but functional MRI (fMRI) has emerged as a useful means to objectify brain response to painful stimulation. We have investigated the effect of chondroitin sulfate (CS) on brain response to knee painful stimulation in patients with knee osteoarthritis using fMRI.

Methods. Twenty-two patients received CS (800mg/day) and 27 patients placebo, and were assessed at baseline and after four months of treatment. Two fMRI tests were conducted in each session by applying painful pressure on the knee interline and on the patella surface. The outcome measurement was attenuation of the response evoked by knee painful stimulation in the brain.

Results. fMRI of patella pain showed significantly greater activation reduction under CS compared with placebo in the region of the mesencephalic periaqueductal gray. The CS group, additionally showed pre/post-treatment activation reduction in the cortical representation of the leg. No effects of CS were detected using the interline pressure test.

Conclusions. fMRI was sensitive to objectify CS effects on brain response to painful pressure on patellofemoral cartilage, which is consistent with the known CS action on chondrocyte regeneration. The current work yields further support to the utility of fMRI to objectify treatment effects on osteoarthritis pain.

Keywords: Functional MRI, knee osteoarthritis, pain, chondroitin sulfate

RESUMEN

Introducción. La artrosis de rodilla es causa de dolor e incapacidad funcional. Uno de los problemas para evaluar la eficacia de los analgésicos ha sido la falta de medidas objetivas de dolor, aunque la resonancia magnética funcional (RMf) ha surgido como un medio útil para objetivar la respuesta del cerebro a la estimulación dolorosa. Hemos investigado el efecto del condroitín sulfato (CS) sobre la respuesta del cerebro a la estimulación dolorosa de la rodilla en pacientes con artrosis mediante RMf.

Métodos. Veintidós pacientes recibieron CS (800mg/día) y 27 placebo y fueron evaluados inicialmente y después de cuatro meses de tratamiento. En cada sesión de RMf se aplicó presión dolorosa sobre la interlínea de la rodilla y en la superficie de la rótula. El resultado se cuantificó como la atenuación de la respuesta cerebral a la estimulación dolorosa de la rodilla.

Resultados. La RMf de la maniobra rotuliana mostró una reducción de la activación en la región de la sustancia gris periaqueductal del mesencéfalo significativamente mayor durante el tratamiento con CS que en la condición de placebo. El grupo de CS, pero no el de placebo, mostró además una reducción de la activación en la representación cortical de la pierna tras el tratamiento. No se observaron efectos del CS con presión dolorosa sobre la interlínea de la rodilla.

Conclusiones. La RMf fue sensible para objetivar los efectos del CS sobre la respuesta del cerebro a la presión dolorosa sobre el cartílago rotuliano-femoral, que es un resultado coherente con la acción conocida del CS sobre la regeneración de los condrocitos. El presente trabajo sugiere nuevamente la utilidad de la RMf para objetivar los efectos del tratamiento en el dolor de origen artrósico.

Palabras clave: RM funcional, artrosis de rodilla, dolor, condroitín sulfato.

INTRODUCTION

The knee is a key element for pedestrian humans to stand and walk. It is the most robust joint in the body supporting nearly the whole weight during erect activity. Nevertheless, the burden of work throughout life favors knee osteoarthritic degeneration, which is a frequent cause of chronic pain and functional disability¹.

A variety of treatments have been tested to alleviate knee osteoarthritis symptoms, most being focused on reducing pain through analgesic or anti-inflammatory actions. Of particular interest, however, are agents aiming at improving patients' clinical situation by interfering with the progression of structural changes in joint tissues. Clinical studies have reported a beneficial effect of pharmaceutical-grade chondroitin sulfate (CS) on knee pain, and a parallel small but significant reduction in the rate of decline in joint space width²⁻⁶. Nevertheless, not all clinical trials have been successful⁷.

Inherent problems with efficacy assessment of pain medication are the lack of objective pain measurements and the large variability of subjective pain ratings⁸.

Noninvasive neuroimaging has emerged as a useful means to objectify brain response to painful stimulation. In particular, functional magnetic resonance imaging (fMRI) has proved its ability to comprehensively map brain activity associated with pain experience⁹. Although there are only few imaging studies on knee osteoarthritis¹⁰⁻¹³, previous work has already characterized brain activity associated with evoked pain, spontaneous pain^{10,14} and pain modulation¹¹ in knee osteoarthritis patients. Two studies have specifically tested analgesic treatment in knee osteoarthritis using lidocaine patches¹³ and single-dose naproxen¹². Interestingly, in both studies, the treatment effect on brain activity attenuation was more evident than on subjective pain score reduction, suggesting the potential usefulness of fMRI to complement the testing of drug effects on pain.

Within the anatomically complex knee, the medial tibiofemoral articular interline is one of the most tender points¹⁵. Pressure on this site in patients with knee osteoarthritis may generate pain from damage or sensitization in a variety of structures (e.g., lateral ligament, joint capsule, synovium, outer edge of the internal meniscus and subchondral bone)¹⁶. Thus, stimulating this

point using focal pressure is a highly sensitive maneuver to elicit pain¹⁵. On the other hand, the pain generated by pressing down the patella surface in osteoarthritic knees is probably less complex, and may be more selectively related to sensitization processes in the bone and the junction between the bone and cartilage as a result of erosion in the patella and femoral cartilages^{16,17}. Agents like CS potentially may improve pain generated in both knee sites, but patella manipulation could be more suitable to identify treatment actions on the cartilage.

The aim of the present fMRI study was to objectively identify the effects of four-month CS treatment on the brain response to pressure painful stimulation in patients with symptomatic knee osteoarthritis. We hypothesized that attenuation of the response evoked in the pain-processing brain system under knee pressure would be a sensitive outcome measurement to capture CS effects on knee osteoarthritic pain.

METHODS

Study population

The current study was developed in the Rheumatology Department and the MRI Research Unit of the Hospital del Mar in Barcelona, from December 2010 to January 2013. Patients attended the Hospital del Mar's services or referred to it from primary health care centers. A total of 78 patients with radiological grade II or III¹⁸ and clinical osteoarthritis based on the American College of Rheumatology (ACR) criteria¹⁹ were screened from whom 64 were randomized (32 to placebo and 32 to CS) (see Suppl. file for eligibility and exclusion criteria and for sample size assumptions). Thirteen patients dropped-out of the study and two more were excluded from the analysis. Finally, 49 patients were evaluable, including 27 in the placebo group and 22 in the CS group (see Figure 1 for patient flow diagram and Table 1 for patient characteristics)^{20,21}.

Written informed consent was obtained from all the patients. The study was approved by the local Ethics Committee (Clinical Research Ethical Committee-Institut Municipal d'Assistència Sanitària (CEIC-IMAS), Barcelona), and in compliance with the World Medical Association's Code of Ethics (Declaration of Helsinki). The authors confirm that all ongoing and related trials for this drug are registered".

Trial design

This study was a randomized, double-blind, placebo-controlled trial (ClinicalTrials.gov. Identifier: NCT01226615) of 120-day treatment with CS once-daily doses of 800 mg (Condrosan, Bioibérica S.A.) versus placebo (see Suppl. file for randomization procedure). CS is a prescription drug containing highly purified chondroitins 4 and 6 sulfate of bovine origin in a concentration of not less than 98%. It has an average molecular weight of ~15-16 kDa, and an intrinsic viscosity of ~0.02-0.06 m³/kg. The primary outcome measurement was attenuation of the response evoked by knee painful stimulation in the pain-processing brain system assessed by fMRI. All patients underwent a baseline pre-treatment fMRI session (day 0) and a final post-treatment fMRI session (day 120, with a window of 14 days). Following screening, eligible subjects underwent a medication wash-out, according to exclusion criteria, prior to the first fMRI assessment. Both CS and placebo were administered in capsule form and were identical in both appearance and

administration method. Paracetamol was available as oral analgesic rescue medication to be taken as required during the study up to a maximum daily dose of 3 g. Subjects were not allowed to take rescue medication 24 h prior to baseline and final fMRI sessions. No important changes to methods after trial commencement were conducted.

Stimulus intensity and study tasks

During the baseline clinical assessment, patients rated the severity of pain for left and right knees on a numerical rating scale (NRS) (0: “No pain”; 10: “Extreme pain”). The most severe knee was used in both baseline and final sessions. The proportion of right/left selected knee in the placebo group was 18/9, and in the CS group 13/9 ($p = 0.767$).

In each fMRI session, brain response to painful pressure on the selected knee was assessed using both the Knee Interline Pressure Test and the Patella Pressure Test. Pressure stimulation was applied using a home-developed MRI-compatible algometer^{22,23}. For the Knee Interline Pressure Test, a pressure surface head of 1cm^2 was used, and for the Patella Pressure Test it was 7.1cm^2 . Painful stimulus intensity (Kg) to be applied in both baseline and post-treatment fMRI sessions was individually adjusted for each patient prior to baseline fMRI. For both tests, we determined the pressure necessary to provoke subjective pain in the range of 5 to 8 in an 11-point NRS by applying the stimulus during 10 sec. During knee interline stimulation, mean ($\pm$ SD) subjective pain attained was 6.8 (0.4) points in the placebo group and 6.7 (0.5) points in patients receiving CS ($t=0.6$ and $P=0.575$), and mean applied pressure was of 2.5 (1.1) Kg and 2.4 (1.4) Kg ($t=0.2$ and $P=0.831$) in placebo and the CS groups, respectively. These data indicate complete group matching for stimulus intensity and evoked pain. During patella pressure, mean pain score was 6.7 (0.7) points in the placebo group and 6.7 (0.6) points in CS group ($t=0.0$ and $P=0.985$), obtained with 4.5 (1.7) Kg in the placebo group and 3.8 (1.5) Kg in the CS group ($t=1.5$ and $P=0.133$). These data also indicate a proper stimulus intensity selection, but the CS had a subtle (non-significant) tendency to need less pressure to generate similar pain scores.

Knee Interline Pressure Test. During fMRI, pressure stimulation was applied on the medial articular interline of the selected knee at the tenderest point in each subject. The pressure was exerted with the knee in the position of 60 degree flexion. The tender point was established by palpation on the anatomical region, it was then marked and snapshots were taken to assist

stimulation site reposition. The test was carried-out during the acquisition of a 6-min fMRI sequence in which 11 rest periods of 20 seconds (plus a final rest period of 30 seconds) were alternated with 11 painful stimulation periods of 10 seconds (Figure 2). Each subject was asked to rate the subjective pain actually perceived during the whole fMRI sequence immediately after fMRI acquisition using NRS.

Patella Pressure Test. Pressure was vertically applied on the patella surface of the selected knee at the patella central point with the knee in an extended (flat) position. Reposition of stimulation site was similarly assisted by a set of snapshots taken at baseline. As in the interline, the test identically alternated rest and stimulation blocks during 6 min and each patient rated subjective pain actually perceived during the experiment.

fMRI parameters. A 3T MRI scanner (Achieva X series, Philips Medical Systems, Eindhoven, The Netherlands) equipped with an eight-channel phased-array head coil and single-shot echo planar imaging (EPI) software was used. Functional sequences consisted of gradient recalled acquisition in the steady state (time of repetition [TR], 2000ms; time of echo [TE], 35ms; pulse angle, 90°; field of view [FOV], 23cm; 96 x 69-pixel matrix; slice thickness, 4mm (plus inter-slice gap, 1mm)). Twenty-two interleaved slices, parallel to the anterior-posterior commissure line, were acquired to cover the brain. The acquisitions were preceded by 4 additional dummy images allowing the pain MRI signal to reach equilibrium.

Statistical analysis

Behavioral analysis. Baseline clinical characteristics were compared between the two groups using Student t tests in the case of quantitative variables and the Chi² tests was used to compare the distributions of categorical variables. Student t tests were also used to compare subjective pain ratings during fMRI testing.

fMRI analysis. All fMRI data were processed using the Statistical Parametric Mapping (SPM8) package, Wellcome Department of Imaging Neuroscience [<http://www.fil.ion.ucl.ac.uk/spm/>], running in Matlab 7.1. Images were realigned, normalized to Montreal Neurological Institute

(MNI)-space (voxel size = $2 \times 2 \times 2 \text{ mm}^3$) and smoothed with a full width at half maximum (FWHM) Gaussian kernel of 8 mm.

Single-subject (1st-level) SPM contrast images were estimated comparing the “pain” condition with the “rest” condition. fMRI signal response at each voxel was modeled with separate regressors and a response delay of 4 sec was considered. In two previous studies using similar procedures^{22,23}, we observed that the duration of brain response to a 10-second pressure painful stimulation approaches 16 seconds. Thus, in this analysis, each block was modeled using a pain condition of 16-sec duration (Figure 2).

The resulting first-level contrast images were then carried forward to subsequent 2nd-level (group) analyses. One-sample t-statistic maps were calculated to describe task-related activations, and ANOVA (SPM Full Factorial repeated measurements within groups and independent between groups) was used to identify group by session treatment interaction effects and to compare groups and sessions.

Thresholding criteria. Results are reported as clusters Family-Wise Error (FWE) corrected for multiple comparisons using an empirical permutation procedure. Spatial extent thresholds were determined by 2,000 Monte Carlo simulations using AlphaSim²⁴ as implemented in the SPM REST toolbox²⁵. The input parameters to AlphaSim included an individual voxel threshold probability of 0.01, cluster connection radius of 5 mm, 8 mm FWHM smoothness, incorporating a mask volume encompassing the whole pain-processing network (70,863 voxels). This mask corresponded to the largest activation map identified in baseline within-group effects (one-sample t-test, including voxels with $p < 0.01$, placebo group, Knee Interline Pressure Test). The estimated minimum cluster size extent was 147 voxels in order to satisfy a FWE rate correction of $P_{\text{FWE}} < 0.05$. Based on this estimate, clusters greater than 147 voxels with $P < 0.01$ were considered significant (FWE-corrected $P < 0.05$) to identify treatment effects.

RESULTS

Subjective pain ratings

Knee Interline Pressure Test. Painful stimulation during fMRI testing produced a similar amount of subjective pain in both placebo and CS groups at baseline and there was no significant treatment effect (Table 2).

Patella Pressure Test. Pressure on the patella during fMRI generated slightly but significantly more subjective pain at baseline in patients latter receiving CS, suggesting a slightly more severe patellar process in this group at the starting point. We found no significant treatment effect. In the CS group, however, a 0.7 points pre/post-treatment pain score reduction was observed, which had a tendency to be significant ($p = 0.077$) with a mild-to-moderate effect size of 0.5 (0.3 in placebo).

Overall, subjective pain analysis indicates that no significant intervention effect was demonstrated using subjective pain scores, but changes were found in the expected direction for the Patella Pressure Test.

Functional MRI

Knee Interline Pressure Test. Group brain activation (one-sample t-test) was robust and involved the whole pain-processing network in both groups and both sessions (See Suppl. Figure 1 and Suppl. Table 1). Nonetheless, we found no significant interaction or session effect and no between-group difference in this analysis.

Patella Pressure Test. Painful pressure on the patella similarly activated the relevant regions of the pain-processing network (one-sample t-test) in both study groups (Figure 3 and Suppl. Table 2).

Brain activation, however, was more extensive in the CS group at baseline (See Suppl. Figure 2 and Suppl. Table 3), which is consistent with between-group differences identified in baseline subjective pain scores.

Group (placebo/CS) by session (pre/post-treatment) interaction was significant showing a larger activation reduction in the CS group than in placebo in a posterior mesencephalon region

including the periaqueductal gray (PAG) (Figure 4). Pre/post-treatment comparison confirmed the direction of this effect by showing activation reduction in the CS group only. All the voxels of the PAG region with significant interaction (Suppl. Table 2) showed a pre>post-treatment difference at $p < 0.05$ (peak difference at $x = -10$, $y = -34$, $z = -16$; $t = 2.4$, $p = 0.007$). In this paired analysis, the CS group showed significant activation reduction in the primary somatosensory cortex (including the cortical representation of the leg) and extending to the primary motor cortex and posterior supplementary motor area (SMA). Group by session interaction consistently revealed a tendency for this cortical change to be larger in the CS than in placebo (peak interaction $x = 2$, $y = -6$, $z = 72$; $t = 2.96$, $p = 0.002$ and 43 voxels -subthreshold- with $p < 0.01$). There were no other treatment effects in this analysis, but between-group differences identified in baseline brain activation (CS activation > placebo activation) were no longer significant after treatment.

When the analyses were repeated considering the intention-to-treat sample (which includes two additional patients [Figure 1 Flow diagram]), we found similar results. No treatment effect was demonstrated for the Knee Interline Pressure Test. For the Patella Pressure Test, a significant group by session interaction with larger activation reduction in the CS group in the PAG region ($x = 0$, $y = -40$, $z = -18$; $t = 2.9$, 159 voxels) and a significant activation reduction in the primary somatosensory cortex in the CS ($x = 0$, $y = -6$, $z = 70$; $t = 2.8$, 409 voxels) were again identified.

DISCUSSION

The aim of the present study was to objectively capture the effects of four-month CS treatment on the brain response to knee painful stimulation in patients with knee osteoarthritis using fMRI. Two different tests were conducted by applying painful pressure on a knee tender point and by targeting the patellofemoral cartilage by pressing the patella down. The study succeeded in the primary objective as a significant effect was demonstrated showing attenuation of brain response to painful pressure in key regions of the pain-processing network using the patella test.

Painful pressure on the knee medial interline provided no positive results. This test generated similar subjective pain in both study groups and no treatment effect was identified with clinical measurements (no significant results, no effect tendencies). The test produced robust pain-processing network activation in both groups, but also no treatment effect was identified with fMRI. Clinical and imaging data, therefore, were consistent each other in showing no treatment effects.

During the patella test, by contrast, study groups showed different responses. Patients receiving CS reported higher subjective pain scores in response to patella pressure at baseline, suggesting that the patellar process was slightly more severe in this group. CS patients coherently showed significantly larger brain activations in this basal session. On the other hand, although the treatment effect on subjective pain rating did not reach the significance level, clinical changes were in the expected direction and showed a tendency to pain reduction after treatment in the CS group with a moderate effect size. In this context, relevantly, fMRI measurements were sensitive enough to identify a significant treatment action. The CS effect on the patella pressure response was captured by a significant group by session interaction involving a brainstem region that includes the PAG, and a significant pre/post-treatment difference involving the cortical representation of the leg. The PAG and the somatosensory cortex both participate in basic steps of nociceptive stimuli processing²⁶. The PAG is a gate through which peripheral pain signals enter the brain and a primary stage where the inputs are modulated via bottom-up and top-down mechanisms^{27,28}. The role of the somatosensory cortex in the processing of pain is also basic, and it mainly relates to spatially localize the injured body site^{29,30}. Overall, the CS effect on primary steps

of brain response to knee painful stimulation is compatible with the event of reducing pain peripheral sources from changes in the injured joint, on where the CS putatively acts.

The most known action of CS is the protective effect on the cartilage^{2,31}. In chondrocytes, CS reduces inflammation cellular events, is pro-anabolic and anti-catabolic with a net increase in collagen and proteoglycan synthesis, and shows anti-apoptotic properties³²⁻³⁵. Chondroitin sulfate also has a direct action on subchondral bone osteoblasts/osteoclasts balance with ultimate reduction of bone resorption³⁶. A significant effect on the patella-related pain may be an expression of the broad CS cartilage protection, as cartilage damage potentially is a relevant contributor to pain generated by patella pressure^{16,17}.

We should, however, be cautious before generalizing our results as a relatively small clinical sample was evaluated. To this end, this study may be contemplated as a test of the potential usefulness of fMRI showing encouraging results that may well emphasize the advantage of incorporating neuroimaging tools for assessing the effect of drugs on pain. Another study limitation is that only pharmaceutical-grade CS was used and, therefore, our results cannot be generalized to other chondroitin sulfate products such as those available in some countries as dietary supplements.

CONCLUSIONS

fMRI was able to objectify CS effects on brain response to knee pressure painful stimulation. Overall, clinical and imaging data were consistent each other, but fMRI demonstrated to be more sensitive. Chondroitin sulfate effect was detected on pain elicited by patellar pressure, where the cartilage component of pain is putatively a relevant factor. This effect is consistent with the CS action on chondrocyte regeneration.

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Other financial interests: Dr. Monfort is a consultant to Bioiberica and has also received Grants from Pfizer and Lacer. Dr. Vergés, Dr. Herrero and Dr. Sánchez are staff of the Research Department of Bioiberica and they have participated in this study as academic researchers. Authors Pujol J, Contreras-Rodríguez O, Llorente-Onaindia J, López-Solà M, Blanco-Hinojo L, Ortiz H, Montañés FJ, Deus J, Benito P each declare: Conflict of Interest: None

Ethical standards

Written informed consent was obtained from all the patients. The study was approved by the local Ethics Committee (Clinical Research Ethical Committee-Institut Municipal d'Assistència Sanitària (CEIC-IMAS), Barcelona), and in compliance with the World Medical Association's Code of Ethics (Declaration of Helsinki).

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Table 1. Clinical characteristics of the study populations

	Chondroitin S. (n=22)	Placebo (n=27)	T/Chi ²	P
Age, mean±SD years	63.7±7.6	65.6±6.2	-0.9	0.346
Gender, female/male	19/3	18/9	2.5	0.111
White race, number	22	27		
Recruitment, PHC/Hospital Units	19/3	19/8	1.8	0.182
Knee pain severity at baseline (NRS)	6.2±1.6	6.4±1.3	-0.5	0.651
Radiological grade, II/III, number	16/6	21/6	0.2	0.683
NSAIDs regular use, number	11	11	0.4	0.517
Lequesne at baseline, mean±SD	12.4±3.0	11.7±3.6	0.7	0.471
SF-36- Physical Component	34.09±7.12	36.5±8.64	-1.1	0.299
SF-36- Mental Component	44.04±13.08	51.10±11.55	-2.0	0.051
Rescue medication, Yes/No	21/1	24/3	0.7	0.404
Averse Events, number of patients	8	10	0.0	0.961

PHC: Primary Health Care. NRS,; 11-point numerical rating scale; NSAIDs: nonsteroidal antiinflammatory drugs; Lequesne Algofunctional Index; SF-36, Medical Outcomes Study 36-item Short-Form General Health Survey.

Table 2. Subjective pain ratings during fMRI testing

	Placebo (n= 27)	Chondroitin Sulfate (n= 22)		
	Mean (SD)*	Mean (SD)*	t	P
Knee Interline Pressure Test				
Baseline (day 0)	7.2 (1.1)	7.6 (1.3)	1.2	0.228
Post-Treatment (day 120)	6.9 (2.4)	7.1 (1.5)	0.4	0.674
Difference from Baseline (pain score reduction)	0.3 (2.3)	0.5 (1.7)	0.3	0.789
Patella Pressure Test				
Baseline (day 0)	6.9 (1.6)	7.8 (1.5)	2.0	0.048
Post-Treatment (day 120)	6.4 (2.4)	7.1 (2.4)	0.9	0.355
Difference from Baseline (pain score reduction)	0.4 (2.4)	0.7 (1.8)	0.4	0.678

11-point Numerical Rating Scale (NRS) scores.

Figure 1. CONSORT Flow Diagram.

Figure 2. fMRI paradigms for knee pressure tests. A, Block design alternating rest and pressure stimulation. B, Blue (rest) & red (activation) lines correspond to the SPM conditions considered to estimate and model the data. C, Home-made algometer to apply pressure on the knee.

Figure 3. Brain activation (one-sample t-tests) obtained during Patella Pressure Test for both groups and conditions. The test generated a robust activation of the entire pain-related brain system. Data are displayed showing $P_{\text{FDR-corrected}} < 0.05$. Right in axial and coronal views corresponds to the right hemisphere.

Figure 4. Chondroitin sulfate effects on brain response to knee painful stimulation during the Patella Pressure Test. Significant group by session interaction (top-left brain views) involved periaqueductal gray (PAG) and surrounding brainstem. Bottom-left sagittal view shows the interaction results at $p < 0.05$. Significant pre > post-treatment differences in the chondroitin sulfate group (top-right brain views) involved the cortical representation of the leg. Bottom-right sagittal view shows pre-post difference at $p < 0.05$, which confirm the direction of the group by session effect at the PAG with activation attenuation in the chondroitin sulfate group.