Naproxen Effects on Brain Response to Painful Pressure Stimulation in Patients with Knee Osteoarthritis: A Double-blind, Randomized, Placebo-controlled, Single-dose Study

Mónica Giménez, Jesús Pujol, Zahid Ali, Marina López-Solà, Oren Contreras-Rodríguez, Joan Deus, Héctor Ortiz, Carles Soriano-Mas, Jone Llorente-Onaindia, and Jordi Monfort

ABSTRACT. Objective. The aim of our study was to investigate the effects of naproxen, an antiinflammatory analgesic drug, on brain response to painful stimulation on the affected knee in chronic osteoarthritis (OA) using functional magnetic resonance imaging (fMRI) in a double-blind, placebo-controlled study.

Methods. A sample of 25 patients with knee OA received naproxen (500 mg), placebo, or no treatment in 3 separate sessions in a randomized manner. Pressure stimulation was applied to the medial articular interline of the knee during the fMRI pain sequence. We evaluated subjective pain ratings at every session and their association with brain responses to pain. An fMRI control paradigm was included to discard global brain vascular effects of naproxen.

Results. We found brain activation reductions under naproxen compared to no treatment in different cortical and subcortical core pain processing regions ($p \leq 0.001$). Compared to placebo, naproxen triggered an attenuation of amygdala activation ($p = 0.001$). Placebo extended its attenuation effects beyond the classical pain processing network ($p \leq 0.001$). Subjective pain scores during the fMRI painful task differed between naproxen and no treatment ($p = 0.037$). Activation attenuation under naproxen in different regions (i.e., ventral brain, cingulate gyrus) was accompanied by an improvement in the subjective pain complaints ($p \leq 0.002$).

Conclusion. Naproxen effectively reduces pain-related brain responses involving different regions and the attenuation is related to subjective pain changes. Our current work yields further support to the utility of fMRI to objectify the acute analgesic effects of a single naproxen dose in patients affected by knee OA. The trial was registered at the EuropeanClinicalTrials Database, "EudraCT Number 2008-004501-33". (J Rheumatol First Release Oct 1 2014; doi:10.3899/jrheum.131367)

Key Indexing Terms:
OSTEOARTHRITIS PAIN NAPROXEN MAGNETIC RESONANCE IMAGING

Knee osteoarthritis (OA) is a degenerative joint disease causing symptoms in 12% of people over the age of 65[1]. The focus of therapy for knee OA includes effective symptomatic control of pain and improvement in health-related quality of life. The currently available therapies do not provide effective control of pain for all

From the MRI Research Unit, CRC Mar; Rheumatology Department, Hospital del Mar; Centro Investigación Biomédica en Red de Salud Mental, CIBERSAM G21; Department of Clinical and Health Psychology, Autonomous University of Barcelona; Bellvitge Biomedical Research Institute, Psychiatry Department, Bellvitge University Hospital, CYBERSAM; Cell Research Group in Inflammation and Cartilage, Institut Hospital del Mar d'Investigacions Mèdiques, Barcelona, Spain; Neurosciences Centers of Excellence for Drug Discovery (CEDD), GlaxoSmithKline, London, UK; Pfizer, Neusentis, Cambridge, UK; Department of Psychology and Neuroscience, University of Colorado, Boulder, Colorado, USA.

Supported in part by the Ministry of Science and Innovation of Spain (Grant SAF2010-19434) and by the Neuroscience CEDD, GlaxoSmithKline. M. López-Solà and J. Deus are part of the Research Group SGR 1450 from the Agency of University and Research Funding Management of the Catalan Government. M. López-Solà is supported by the postdoctoral fellowship Beatriu de Pinós, awarded by the Agency for the Management of University and Research funding (Agència de Gestió d'Ajuts Universitaris i de Recerca; AGAUR) from the Catalan Government (2010 BP_A 00136). Dr. Soriano-Mas is funded by a Miguel Servet contract from the Carlos III Health Institute (CP10/00604).

M. Giménez, PhD; O. Contreras-Rodríguez, PhD; H. Ortiz, MsC, MRI Research Unit, CRC Mar, Hospital del Mar; J. Pujol, MD, MRI Research Unit, CRC Mar, Hospital del Mar, Centro Investigación Biomédica en Red de Salud Mental, CIBERSAM G21; Z. Ali, PhD, Neurosciences CEDD, GlaxoSmithKline; M. López-Solà, PhD, MRI Research Unit, CRC Mar, Hospital del Mar, Department of Psychology and Neuroscience, University of Colorado; J. Deus, PhD, MRI Research Unit, CRC Mar, Hospital del Mar, Department of Clinical and Health Psychology, Autonomous University of Barcelona; C. Soriano-Mas, PhD, Bellvitge Biomedical Research Institute-IDIBELL, Psychiatry Department, Bellvitge University Hospital, CIBERSAM; J. Llorente-Onaindia, MsC; J. Monfort, MD, PhD, Rheumatology Department, Hospital del Mar, Cell Research Group in Inflammation and Cartilage, Institut Hospital del Mar d'Investigacions Mèdiques.

Address correspondence to Dr. J. Pujol, Department of Magnetic Resonance, CRC-Mar, Hospital del Mar, Passeig Marítim 25-29, 08003, Barcelona, Spain. E-mail: 21404jpn@comb.cat

Accepted for publication July 24, 2014.

patients. Therefore, there is much interest in developing effective and well-tolerated medications that can reduce pain. A key limitation in the development of novel analgesic treatments is the early assessment of therapeutic efficacy. This is at least partly related to the variability in quantifying subjective pain responses[2], which may hinder the detection of significant differences between groups. In addition, clinical trials investigating analgesic efficacy suffer from profound placebo effects that may mask the magnitude of any analgesic efficacy[3,4]. As a consequence, there is significant interest in developing more objective measures of potential analgesic efficacy in pain.

The field of pain research has progressed exponentially along the last decade owing to the advancement of non-invasive brain techniques. Functional magnetic resonance imaging (fMRI) demonstrates considerable anatomical resolution when mapping specific regions of the brain, including areas collectively termed as the "pain matrix" that seem to appear critical for generating a pain experience[5,6]. The fMRI brain signature of pain experience involves a nociception sensory component related to brain activity in the thalamus and primary/secondary somatosensory cortices, an effective component related to the insula and anterior cingulate cortex, and a cognitive-evaluative component mostly implicating the frontal cortex[7,8,9,10,11,12,13]. The amygdala and other subcortical structures [e.g., periaqueductal gray (PAG)] are critical to pain processing as response modulators. Amygdala activation is not always present in provocation studies, but it may be evident, for instance, during pain anticipation[3] or during attention to pain unpleasantness[10].

The assessment of brain response to painful stimulation may be of major clinical and social effect in chronic conditions because they remain poorly understood and insufficiently treatable by existing therapies[14,15]. Imaging studies show that the brain reorganizes in relation to chronic pain, in a pattern specific to the type of clinical pain. For example, relevant studies have demonstrated that chronification of back pain shifts brain representation of spontaneous pain from sensory to affective-cognitive circuits[16]. In the few imaging studies in patients with knee OA, brain activity associated with spontaneous pain also seems to be notably modified in patients with chronic OA, but evoked pain by knee pressure appears to involve brain regions commonly observed for acute pain[17,18]. A study showed an abnormal interference effect of painful stimulation on attention-related brain areas in patients with chronic knee OA[19]. Finally, 2 preliminary studies that assessed the brain effects of analgesic drugs on joint pain in knee OA (lidocaine in 5 patients[20] and valdecoxib in 6 patients[17]) suggest the potential usefulness of the imaging tools in treatment evaluation.

The goal of our fMRI study was to objectively identify the effects of a classical pharmacological intervention in OA, naproxen (single dose), on the brain response to mechanical painful stimulation applied to the most affected knee in patients with chronic OA. fMRI has the potential to improve decision making in the process of developing new treatment drugs[21], so the use of a single-dose model assessing the response patients with OA to antiinflammatory treatment may provide a simple, early, and valuable guide for future studies in the clinical drug-development context in OA.

MATERIALS AND METHODS

Trial design. Ours is a placebo-controlled, double-blind, randomized, 3-period crossover study. Following screening, eligible patients underwent a washout treatment period of 7 days (see supplementary material for eligibility criteria, available online at jrheum.org). Patients participated in a total of 3 study sessions involving 500 mg naproxen and placebo, and a session with no treatment (Figure 1). The rationale for including both a placebo and a nontreatment session is that it remains unclear what the magnitude and pattern of the placebo brain response would be in this patient population. The placebo response has been well characterized only clinically[22] and in fMRI studies in healthy populations[3,23]. The patients were told they "would receive a dose of an efficient analgesic in 1 session, no real medication (placebo) with identical appearance in other session, and no medication in other session". No treatment session was only assigned to sessions 1 or 2 to minimize the possibility that patients could identify it as the last session. Patients were assigned to 1 of 4 treatment sequences in accordance with a randomization schedule (A/C/B; B/C/A; C/A/B; C/B/A. A: naproxen 500 mg, B: placebo, C: no treatment). Independent pharmacists dispensed the pills to the investigator according to the randomization list, while the investigators and the patients remained blind to the treatment assignment. Both naproxen and placebo were administered in capsule form, and were identical in appearance and method of administration. For the no-treatment session, an identical jar was dispensed to the subject, but no capsule was inside. The trial was registered at the European Clinical Trials Database, "EudraCT Number 2008-004501-33".

Study population. Our current study took place at the Hospital del Mar in Barcelona in collaboration with additional primary healthcare centers associated to the hospital (16 centers) and 2 external monographic offices of controlled patients with OA. Initially, 127 patients were called, from whom 79 were successfully localized and 48 agreed to participate in our study. From the 48 patients, 27 passed the screening period while the remaining 21 failed to pass the screening tests; they did not come to the initial visit to be enrolled or they decided not to participate. See supplementary material for sample size rationale (available online at jrheum.org).

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

Interventions: treatments. Patients received oral naproxen 500 mg (nonsodium form of naproxen, Naproxyn EC, Roche Products Ltd.), placebo, or no treatment in each of the 3 separate sessions and were dosed in the unit. fMRI assessments were fixed to start at 4 h post-dosing and stop by 5 h post-dosing (after peak plasma concentration of naproxen, 2–4 h). Patients were asked to consume a light breakfast at home prior to attending the unit and were provided with a light meal 2 h following dosing. Between treatment sessions, paracetamol (1000 mg) was available as an oral analgesic rescue medication to be taken as required at any time during our study (up to a maximum daily of 4 g), with the exception of the period between the 12 h prior to the fMRI scanning and the 5 h post-dose. Patients were told not to leave the hospital, to avoid any extra exercise.

Interventions: subjective pain assessments. During each of the 3 visits, patients were required to rate pain on an 11-point numerical rating scale

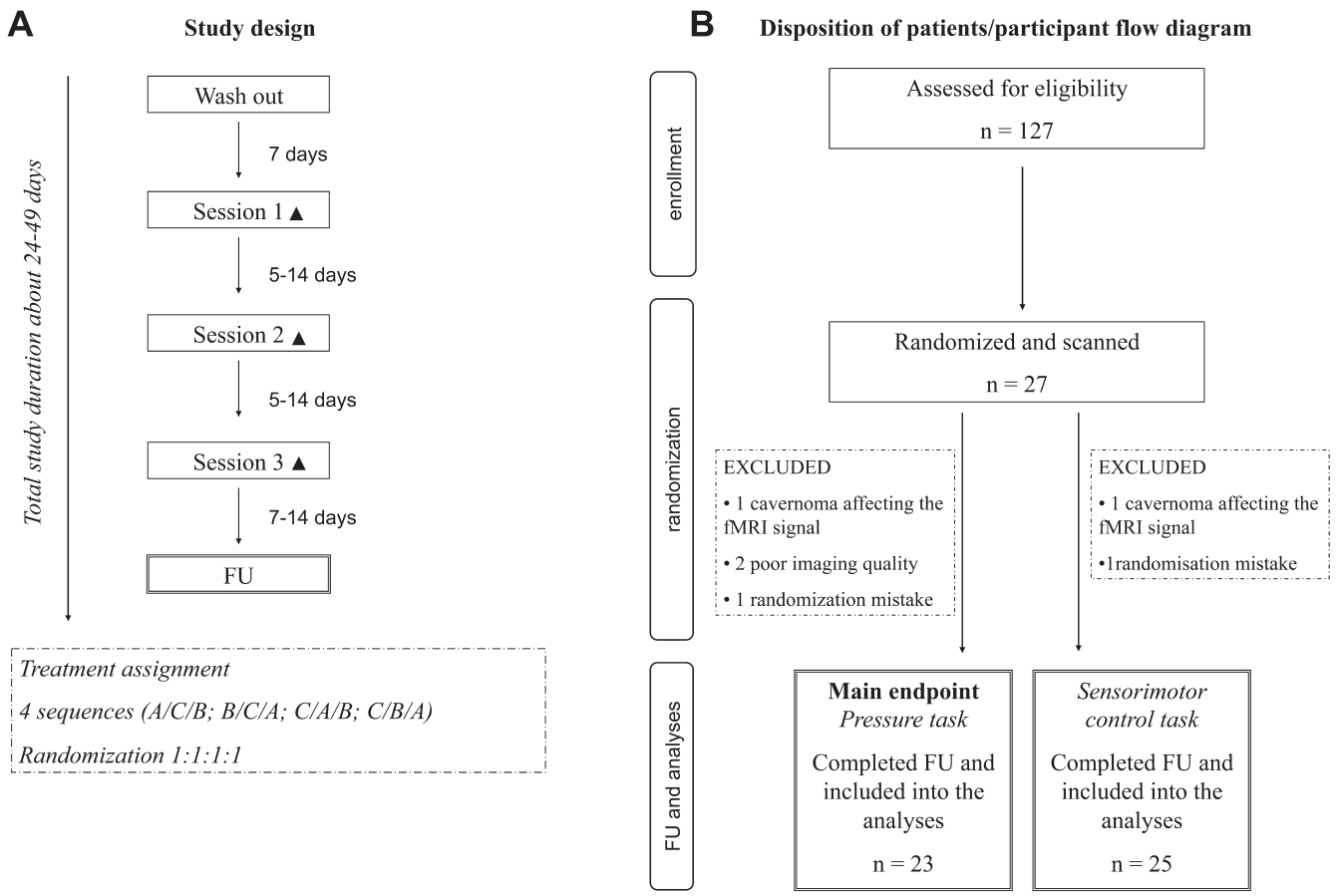


Figure 1. Flow diagram: study design and disposition of patients. (A) Study design. Patients went through 3 scanning sessions having as a primary endpoint the evaluation of fMRI sensitivity to detect pain from mechanical stimulation by means of pressure stimuli of the OA knee following treatment with a single dose of naproxen compared to placebo. Four possible randomization sequences were used. (B) Disposition of patients. A = naproxen 500 mg, B = placebo, and C = no treatment. ▲: NRS measures around the pressure pain in 4 timepoints within a session: predose, prescan, during scan, and postscan. fMRI: functional magnetic resonance imaging; OA: osteoarthritis; FU: followup; NRS: numerical rating scale.

(NRS; 0 corresponding to "no pain" and 10 to "extreme pain") at different timepoints: (1) predose-evoked pain after 10 s of sustained painful stimulation (identical pressure that would be exerted across the fMRI task); (2) postdose/prescan-evoked pain after identical 10 s of sustained painful stimulation; (3) during-scan-evoked pain (during the whole fMRI painful task); and (4) postscan pain after fMRI with no knee manipulation. Additionally, subjective ongoing pain in the affected OA knee was collected at screening visit.

fMRI tasks. The main task consisted of a 6 min 30 s sequence alternating 11 baseline periods of 20 s (plus a final baseline period of 30 s) and 11 painful stimulation periods of 10 s. We applied pressure stimulation on the medial articular interline of the selected knee at the most tender point in each subject with the knee in the position of 60° flexion. The tender point was established by palpation and marked using a permanent felt-tip pen. Pressure painful stimulation was applied using an MRI-compatible algometer developed in-house, with a pressure surface of 1 square cm (Supplementary Figure 1, available online at jrheum.org). Stimulus intensity was individually adjusted for each fMRI assessment to provoke a degree of pain between 5 and 7 in an 11-point NRS. This procedure was repeated each day before drug administration (4 h before fMRI) to account for variable sensitivity from day to day in the painful knee site. Thus, during fMRI assessment, the stimulus intensity applied reflected each subject's sensitivity to painful pressure in the basal situation before dosing.

An additional fMRI non-painful sensorimotor task was included to discard possible global effects on fMRI signal induced by naproxen (see supplementary material for sensorimotor task description, available online at jrheum.org).

fMRI. A 1.5 T Signa Excite system (General Electric) equipped with an 8-channel, phased-array head coil and single-shot echo planar imaging software was used. Acquisition variables and preprocessing procedures are detailed in the supplementary material (available online at jrheum.org).

Analysis. Consistent with previous works[24], we verified that the duration of brain response to a 10-s painful stimulation approaches 16 s (Supplementary Figure 2, available online at jrheum.org). Thus, our analysis was based on a 16-s activation condition for each experimental block. In contrast, "painful-related blocks versus non-painful control blocks" was tested at the individual level using the Statistical Parametric Mapping (SPM) package (Wellcome Department of Imaging Neuroscience). The sensorimotor control task was analyzed as a simple block task comparing the 30-s experimental condition blocks versus the 30-s control condition blocks. Individual images were then included in second-level (group), random-effects analyses. Repeated-measures ANOVA in SPM was used for each fMRI task to compare treatment session effects.

We conducted an additional SPM analysis to correlate the change in subjective pain felt during the fMRI painful task (no treatment pain scores minus naproxen pain scores) with brain maps of the difference in "activation during no treatment minus activation during naproxen".

fMRI thresholds. Results were considered significant when showing p

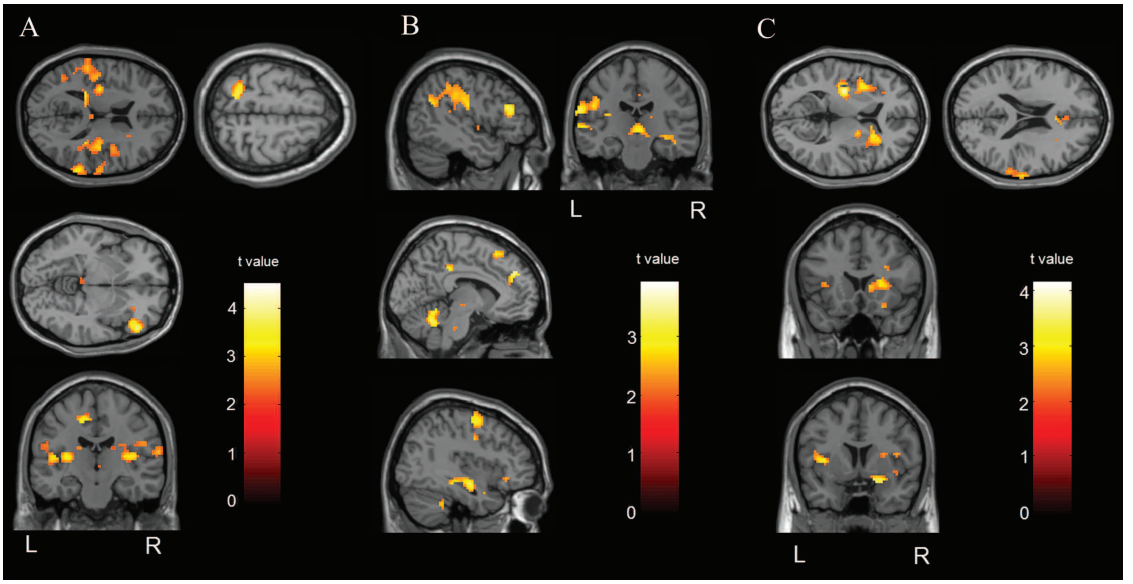
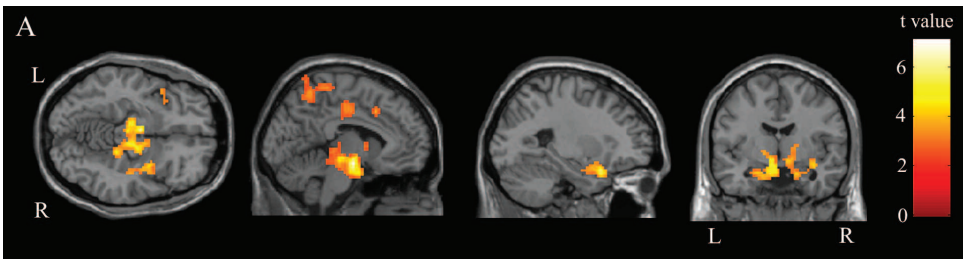


Figure 2. Reduction of brain activity under naproxen and placebo conditions during the fMRI painful pressure task. (A) Naproxen versus no treatment. (B) Placebo versus no treatment. (C) Naproxen versus placebo. Display $p \leq 0.05$, 10 v; results masked by activation pattern under the no-treatment condition. L: left; R: right; fMRI: functional magnetic resonance imaging.



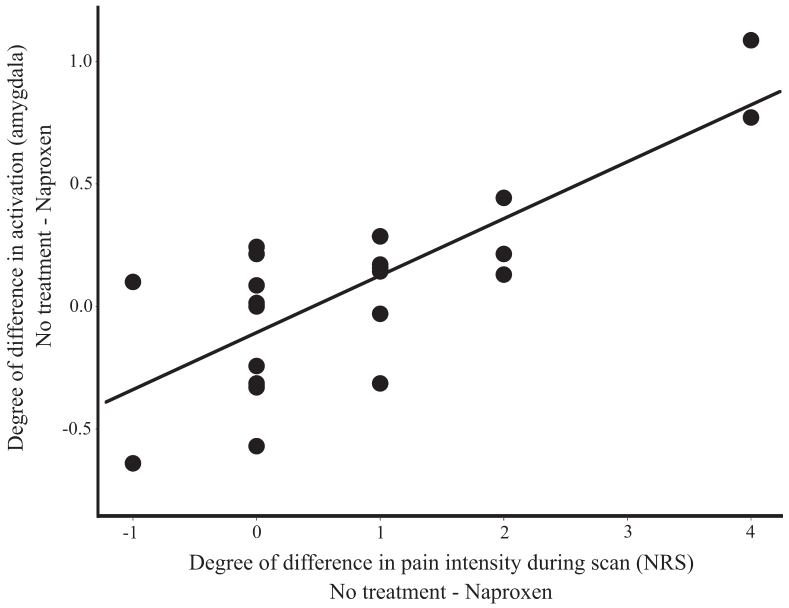


Figure 3. Correlation between brain activation changes and subjective pain perception changes by naproxen. (A) Map illustrating the ventral part of the brain, as well as the cingulate region related to the changes in the subjective perception of pain by treatment. The resultant map identified the regions in which the magnitude of the activation attenuation was related to the attenuation of the perceived pain during painful stimulation (hot colors; "the more the brain activation attenuation, the less the pain perceived"). L: left; R: right. (B) Scatter diagram of the degree of the amygdala activation difference between conditions against the degree of the difference between conditions of the pain perception. Spearman rho = 0.647, $p = 0.001$ for the scatter diagram data. Points represent real data. The line represents data adjusted to the theoretical model. Display $p \leq 0.01$, 10v.

(uncorrected) ≤ 0.001 and a cluster extension ≥ 10 voxels (270 mm^3 each cluster). Within a set of pain-processing network core regions defined *a priori,* treatment effects at $p < 0.01$ (and ≥ 10 voxels) were also reported. *A priori* regions, as identified in a previous study[25], involved primary/secondary somatosensory cortex, insula, anterior cingulate cortex, thalamus, and lateral frontal cortex.

RESULTS

Study population. A total of 27 patients with OA were randomized. While final, good quality fMRI data for the control task were available on 25 patients, for the painful task, 23 patients were analyzed (Figure 1). Sociodemographic characteristics of the patients are summarized in Table 1. There were no treatment-related adverse events.

Brain activation during painful stimulation (group effects). Brain activation during the painful fMRI task for each treatment session is depicted in Supplementary Figure 3 (available online at jrheum.org). Supplementary Figure 3A (available online at jrheum.org) shows the activation pattern during the no-treatment session, reflecting the "baseline" activation pattern. We found significant and consistent activation in the regions belonging to the core pain-processing network. During the no-treatment session, patients demonstrated a strong activation in areas related to the sensory component of nociception (thalamus and primary/secondary somatosensory cortices), the affective component [insula and anterior cingulate cortex (ACC)-supplementary motor area (SMA)], and the cognitive-evaluative component (frontal cortex). Supplementary Figures 3B and 3C (available online at jrheum.org) showed similar, but less strong and extensive patterns for the placebo and the naproxen treatments.

fMRI activation differences between treatment conditions. Table 2 and Figure 2 report the differences in fMRI responses to mechanical painful stimulation to the OA knee between the 3 conditions. Five core, pain-processing network regions implicating all the components of pain experience demonstrated activation reductions under the naproxen compared to no treatment condition [superior parietal region, supramarginal gyrus, posterior insula/secondary somatosensory cortex (SII), ACC-SMA, frontal lateral region]. Compared to placebo, significant activation reductions by naproxen were found in the supramarginal gyrus, insula, basal ganglia, ACC-SMA, as well as in the amygdala. Placebo reduced brain activation in 3 core regions of the pain-processing network (supramarginal gyrus, frontal lateral region, thalamus), extending its effects toward the medial frontal region, the hippocampus, and the cerebellum. The opposite contrasts (naproxen vs no treatment, placebo vs no treatment, and naproxen vs placebo) showed no significant results.

Subjective NRS pain scores. Supplementary Figure 4 (available online at jrheum.org) shows NRS pain intensity scores reported by patients during each condition and timepoint across the experiment (n = 23). Mechanical stimulation of the knee produced identical predose subjective pain in all 3 conditions in terms of group mean (6.91) and SD

Table 1. Main characteristics of our study population. All measurements were obtained at the screening visit, excepting the BMI (having signed the informed consent, the first visit day) and the AE.

Demographics	Patients with OA, n = 25
Age, yrs, mean ± SD (range)	64 ± 7.1 (52–79)
Sex, female/male, n (%)	20 (80)/5 (20)
White, n (%)	25 (100)
Recruitment sites, PHC/ES, n (%)	13 (52)/12 (48)
Baseline pain[a], mean ± SD (range)	6.2 ± 1.1 (4–8)
NSAID regular use, n (%)	25 (100)
Currently depressed, n (%)	2 (8)
Smoking habits, current smoking/not ever smoking, n (%)	0 (0)/2 (8)
Drinking habits, current drinking/not ever drinking, n (%)	0 (0)/1 (4)
BMI, kg/m^2, mean ± SD (range)	29.9 ± 3.2 (24.9–35.3)
Diabetes, n (%)	4 (16)
Controlled diabetes, by treatment/by diet, n (%)	2 (8)/2 (8)
Hypertension, n (%)	13 (52)
Controlled hypertension, by treatment/by diet, n (%)	11 (44)/2 (8)
Cholesterol, n (%)	11 (44)
Controlled cholesterol, by treatment/by diet n (%)	7 (28)/4 (16)
AE during the study, n (%)	2 (8)
Prostatitis, n (%)	1 (4)
Cough, n (%)	1 (4)

[a]Subjective pain at rest felt in the affected OA knee at the screening visit without painful stimulation, rating on an 11-point NRS (from 0 to 10, with 0 corresponding to "no pain" and 10 corresponding to "extreme pain"). BMI: body mass index; AE: adverse events; PHC: primary health care; ES: external services; NSAID: nonsteroidal antiinflammatory drugs; OA: osteoarthritis; NRS: numerical rating scale.

Table 2. Reduction of brain activity by placebo and naproxen. Reductions defined as brain response differences within the contrast: fMRI painful blocks versus control rest blocks. Comparisons between (1) no treatment versus naproxen, (2) no treatment versus placebo, and (3) placebo versus naproxen.

Region of Interest	Peak Coordinates, MNI[a], (Region Volume, mm^3)	T[b]	p[b]
No treatment vs naproxen			
Pain–processing network core regions			
Superior parietal cortex	**–24 / –57 / 63 (1.404)**	**3.58**	**< 0.001**
Supramarginal gyrus	**69 / –42 / 18 (1.134)**	**3.22**	**0.001**
Insula/SII	42 / –18 / 12 (864)	3.08	0.002
Anterior cingulate cortex–SMA	**–15 / –18 / 54 (378)**	**3.16**	**0.001**
Lateral frontal cortex	**54 / 36 / –6 (1.620)**	**3.66**	**< 0.001**
No treatment vs placebo			
Pain–processing network core regions			
Thalamus	3 / –18 / 0 (459)	2.80	0.003
Supramarginal gyrus	**–69 / –24 / 24 (1.269)**	**3.50**	**< 0.001**
Lateral frontal cortex	51 / 27 / –9 (378)	2.82	0.003
Other regions of interest			
Medial frontal cortex	**–12 / 48 / 33 (1.053)**	**3.93**	**< 0.001**
Hippocampus	**39 / –12 / –15 (594)**	**3.09**	**0.001**
Cerebellum	**–12 / –60 / –24 (1.944)**	**3.26**	**0.001**
Placebo vs naproxen			
Pain–processing network core regions			
Supramarginal gyrus	**–33 / –18 / 9 (1.134)**	**4.17**	**< 0.001**
Insula	–42 / 6 / 9 (567)	2.91	0.002
Insula–basal ganglia	24 / 21 / 6 (702)	2.93	0.002
Anterior cingulate cortex–SMA	**6 / 27 / 18 (270)**	**3.88**	**< 0.001**
Other regions of interest			
Amygdala	**21 / 6 / –15 (621)**	**3.25**	**0.001**

Bold face represents regions with significant results at $p \leq 0.001$. [a]x/y/z coordinates (sagittal/coronal/axial) for points showing the greater (peak) difference are given in MNI space. MNI space is based on a standard anatomical brain template that enables the comparison of results obtained across different studies. [b]T and p values for the point showing the greater differences at the implicated region. MNI: Montreal Neurological Institute; SII: secondary somatosensory cortex; SMA: supplementary motor area; fMRI: functional magnetic resonance imaging.

Table 3. Correlation between the pain scores and the fMRI changes. Variables of the correlation defined as "subtraction of the activation map during the no treatment from the activation map during the naproxen" versus "subtraction of the subjective NRS pain scores during the fMRI painful task under no treatment from the subjective NRS pain scores during the fMRI painful task under naproxen".

Region of Interest	Peak Coordinates, MNI[a], (Region Volume, mm^3)	T[b]	p[b]
Large cluster including the ventral striatum, brain stem, amygdala, hippocampus, superior temporal gyrus, insula–operculum, thalamus, middle frontal gyrus, postcentral parietal gyrus	–6 / –9 / –15 (89.235)	7.14	< 0.001
Anterior cingulate cortex	–12 / 18 / 45 (3.240)	4.16	< 0.001
Medial cingulate cortex	–12 / –15 / 30 (11.097)	4.26	< 0.001
Superior frontal gyrus	–9 / 9 / 72 (756)	3.92	< 0.001
Fusiform, parahippocampus	33 / –63 / –9 (2.727)	4.73	< 0.001
Superior parietal lobe	15 / –51 / 60 (14.121)	4.18	< 0.001
Cerebellum	–18 / –36 / –39 (1.323)	3.84	< 0.001

[a]x/y/z coordinates (sagittal/coronal/axial) for points showing the greater (peak) correlation are given in MNI space. MNI space is based on a standard anatomical brain template that enables the comparison of results obtained across different studies. [b]T and p values for the point showing the greater differences at the implicated region. fMRI: functional magnetic resonance imaging; NRS: numerical rating scale; MNI: Montreal Neurological Institute.

(0.3). There were no significant between-conditions differences in the NRS scores during the prescan assessment (F = 2.36, p = 0.103). NRS scores assessing pain perception during the fMRI painful task differed between conditions (F = 3.43, p = 0.038); posthoc Tukey tests indicated that there was a significant difference in pain ratings between naproxen and no-treatment sessions (mean difference 0.83, 95% CI 0.04–1.61, p = 0.037). The mean (SD) values for postscan (no painful stimulus applied) were 1.39 (2.1), 0.91 (1.4), and 1.04 (1.4) for no treatment, placebo, and naproxen, respectively (F = 0.493, p = 0.613).

Correlation between subjective NRS pain scores and fMRI treatment effects. Brain areas where the magnitude of naproxen-induced activation attenuation was associated with significant reductions in subjective pain during the fMRI task are summarized in Table 3 and illustrated in Figure 3. Results involved ventral brain structures related to nociception modulation (e.g., superior pons and midbrain including the PAG and amygdala-hippocampus), but also regions related to the sensory component, such as ventral parts of the insula-operculum and thalamus (Figure 3A). Interestingly, the cingulate cortex also stands out as a relevant region showing the same trend. The scatterplot in Figure 3B illustrates the correlation between amygdala activation reductions and subjective pain decreases from no treatment to naproxen condition.

An additional analysis was conducted to correlate scores of subjective ongoing pain in the affected OA knee collected at screening visit with baseline brain activation and brain activation map of the difference between naproxen and no treatment. Significant correlations were observed in the brainstem and basal ganglia (Supplementary Figure 5, available online at jrheum.org).

Sensorimotor control task. This task was used as a control to discard possible global effects on brain response by naproxen. We found a consistent activation pattern during the sensorimotor task in the visual, the motor, and the auditory cortices when patients were under no treatment (Supplementary Figure 6, available online at jrheum.org). We found no significant suprathreshold clusters when looking for possible activation reductions by naproxen compared to no treatment within the regions triggered by the sensorimotor task.

DISCUSSION

Framed within the recent and promising fMRI application to study drug-induced analgesic effects in chronic pain populations, our current study was able to provide objective measurements of the effects of naproxen, a traditional nonsteroidal antiinflammatory analgesic drug (NSAID), on the brain response to acute pain applied to the painful joint in patients with chronic OA.

Inflammatory pain is produced by nociceptor sensitization in the joint and surrounding tissue, and is enhanced by the sensitization effects of prostaglandin release within the central nervous system. Tissues in the knee containing nociceptors include primarily the joint capsule, ligaments, synovium, bone, and the outer edge of the meniscus[26]. NSAID compounds such as naproxen have a general anti-inflammatory action mainly through cyclooxygenase inhibition, thereby preventing the conversion of arachidonic acid to prostaglandins[27,28]. Naproxen may thus produce analgesia by reducing the nociceptive inputs to brain as a result of its local/peripheral antiinflammatory effect[29,30,31]. On the other hand, reduction of peripheral inflammation may secondarily abort the subsequent central sensitization phenomenon[28].

A direct effect of naproxen on brain prostaglandin system is also probable. Despite the limited central nervous system permeation of total naproxen with a brain/blood ratio of 0.02[32,33,34], the relative concentrations of free (not bound to proteins) naproxen in the brain may compare to free plasma concentrations. Although the ability to inhibit prostaglandins in the central nervous system has not been documented specifically for naproxen, research with other NSAID suggests that this action may be biologically relevant[28]. Thus, naproxen most likely has activities both centrally and peripherally, but which ones are more important is not currently known.

In the context of assessing drug effects on pain, it is important to note that fMRI permits objectifying the experience of pain, which may add a unique value to the clinical trials. In the case of naproxen, a global reduction of the response to the nociceptive stimulus was observed. Brain activation was attenuated in different cortical and subcortical pain-processing regions contributing to different pain domains, that is, the sensory-discriminative dimension (i.e., supramarginal gyrus, superior parietal cortex, posterior insula/SII), but also the cognitive-evaluative (lateral frontal cortex, ACC) and the affective-motivational dimensions (i.e., ACC, insula)[7,8,9,10,11,12,13]. Compared to the placebo treatment session, dosing with naproxen was accompanied by an attenuation of pain-related activation in a part of the network (supramarginal gyrus, insula, basal ganglia, ACC-SMA) and in the amygdala. In our study, a significant naproxen effect was identified using subjective pain rating despite the relatively reduced sample assessed, which may probably be related to the fact that patients were highly trained to rate subjective pain (4 times × 3 sessions). fMRI, however, allowed us not only to objectify the subjective effect, but results showed a complete functional anatomy involving changes at the sensory, affective, and cognitive domains.

Results from the correlation analysis (highlighting the involvement of ventral brain regions) are noteworthy. A prominent region in which activation attenuation under naproxen was strongly correlated with reductions in subjective pain ratings was the brain stem, mainly involving

the mesencephalon and the pons, extending toward the amygdala and the ventral striatum. The pattern illustrates the extent to which the analgesic effect of naproxen is mostly proportional to the changes in deep brain structures related to the modulation of nociceptive processing. Interestingly, such an effect location appears to agree well with a previous pharmacological study of depressive patients by our group, in which dosing with duloxetine was associated with an attenuation of activation in the pons during acute pain linearly associated with an improvement in somatic complaints in such patients[35].

The correlation between pain ratings and fMRI changes implicated the amygdala, which is in contrast with the event of finding no significant baseline amygdala activation. The amygdala is indeed a relevant element in the processing of pain, but its activation is highly variable in pain experiments[36]. Only a small proportion of previous imaging studies have reported significant changes in the amygdala, which involved both signal increases and decreases according to a recent metaanalysis[36]. Thus, the finding of no net amygdala activation during pain provocation in our study is not an unexpected finding. Actually, the amygdala appears to play a dual facilitatory and inhibitory role in the modulation of pain behavior and nociceptive processing that depends on environmental conditions and affective states[37]. This is important in the clinical context because different amygdala activation patterns have been noted between healthy and clinical pain studies[36], and between spontaneous and experimental pain specifically in patients with knee OA[18]. The correlation between pain score reduction and fMRI signal reduction after naproxen may be considered a complementary finding in our study, expressing a distinct modulatory phenomenon in chronic responder patients compared with no responders.

In our study, it is also relevant that we have tested the fMRI brain response to placebo in a clinical population. Few specific imaging studies have been done in patients with chronic OA, even though the placebo effect is notable in this clinical condition[38,39]. Experimental work has shown that placebo analgesia is mediated by the activation of descending, mostly opioids, pain modulatory circuits with the implication of the prefrontal cortex, anterior cingulate cortex, and deep elements like the PAG and amygdala[40,41,42,43,44]. As a result, the brain response to nociceptive stimulation is attenuated[3,45]. In our empirical clinical study, we tested just the outcome of the placebo effect that showed response attenuation in a variety of brain structures related to pain perception. Relevantly, placebo-related changes involved the dorsal lateral prefrontal cortex, which is one of the key areas mediating the top-down effects of placebo[40,41,42,43,44]. Compared with placebo, naproxen showed a stronger effect on core regions of the pain-processing system. Thus, overall, the scenario suggests a significant placebo-related modulation of the pain experience, but with a more specific nociceptive effect of the active treatment. Our study may add a piece of information, but more research is clearly needed to fully disentangle the mechanisms of the placebo effect in the situation of chronic suffering.

Our current study does, however, present some limitations that deserve consideration. If we take into account that the certainty of the pain intensity of an imminent stimulus is considered to be a biological trigger of fear and emotional responses, presenting a dual influence (stress-pain), our correlation approach shows naproxen-related changes affecting the emotional domain, so future studies in OA pain should endeavor to identify the affective emotional state. Also, although our primary analysis was focused on specific areas of the pain matrix based on *a priori* hypotheses, the threshold for significance applied may be considered relatively lenient. The preliminary nature of such observations, and the need for replication in other pain cohorts and with more stringent statistical correction, should be taken into account in the future.

To the best of our knowledge, there are no imaging studies to date assessing the effects of a single naproxen dose on brain responses to pain evoked by pressure stimulation in patients with chronic knee OA. In particular, there are no published studies directly comparing pain ratings or brain responses during the 3 treatment conditions (naproxen, placebo, and no treatment). The use of pharmacological fMRI methodology to objectively evaluate drug action on the brain is well known[46]; in our present study, we further contribute to this idea by specifically addressing brain responses in the context of pain. Our current study provides unique and novel information as to the actual effects of the paradigmatic NSAID naproxen on the modulation of evoked pain responses in the brain (i.e., in the neural systems intimately associated with the final subjective pain experience), and further supports the utility of such a technique in objectifying the acute analgesic effects of the drug in patients affected by knee OA.

ACKNOWLEDGMENT

We acknowledge and thank all the patients for their involvement in the study.

ONLINE SUPPLEMENT

Supplementary data for this article are available online at jrheum.org.

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