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Increased brain responses during subjectively-matched mechanical pain stimulation in fibromyalgia patients as evidenced by MEG.


TITLE: Increased brain responses during subjectively-matched mechanical pain stimulation in fibromyalgia patients as evidenced by MEG.

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Highlights:

- Fibromyalgia patients showed increased brain responses with respect to controls during subjectively-matched painful mechanical stimulation.

- Increased activity was found in somatosensory, temporal and parietal areas at short latencies and in prefrontal areas at both short and long latencies.

- Therefore, central pain augmentation in fibromyalgia is not only present when patients and controls are subjected to the same level of stimulation but also occurs when stimulation is adjusted to produce similar subjective levels of pain in both groups.

ABSTRACT

Objective: The precise pathophysiology of fibromyalgia, a syndrome characterized by chronic widespread pain, remains to be clarified. When subjected to the same amount of stimulation, patients show enhanced brain responses as compared to controls, providing evidence of central pain augmentation in this syndrome. We aimed to characterize brain response differences when stimulation is adjusted to elicit similar subjective levels of pain in both groups.

Methods: Magnetoencephalography (MEG) was used to investigate the brain responses to pressure stimulation applied both above and below the pain threshold in 9 patients and 9 control subjects. A device was developed to deliver pressure pulses in a quantifiable and precise manner. The amount of pressure was adjusted to produce similar subjective pain in both groups.

Results: A between-group comparison of differences between responses evoked by stimulation above and below the pain threshold was performed using cluster-based permutation testing. Increases in signal amplitude in somatosensory, temporal and parietal
areas at short latencies, and in prefrontal areas at both short and long latencies, were found to be larger for patients than for control subjects.

Conclusion: Fibromyalgia patients show enhanced brain responses after reducing the amount of pressure to produce similar subjective levels of pain than to the control subjects.

Significance: The present results suggest that central pain augmentation is present in fibromyalgia, not only when the objective level of stimulation is kept the same as for control subjects, but also when stimulation is adjusted to produce similar levels of pain in patients and controls.

1. INTRODUCTION

Fibromyalgia (FMS) is a syndrome characterized by chronic widespread musculoskeletal pain, mood and sleep disorders and daytime fatigue (Abeles et al., 2007; Merskey and Bogduk, 1994) as defined by the American College of Rheumatology (ACR) 1990 classification criteria (Wolfe et al., 1990). Recently, the use of a pain index and a symptom severity rating have been recommended for diagnosis confirmation. (Wolfe et al., 2010). FMS affects approximately 2% of the population: 3.4% of women and 0.5% of men. (Wolfe et al., 1995).

Studies of experimentally induced pain demonstrate that these patients have a lower pain threshold, as lower intensity stimuli are needed to evoke pain (Price and Staud, 2005). Imaging work provides evidence of central pain augmentation in this condition. An fMRI study has shown that when the amount of stimulation is adjusted to produce similar subjective levels of pain in both patients and control subjects the profile of activated brain areas is similar. In contrast, many more areas are activated in FMS patients when the stimulation level is objectively the same. (Gracely et al., 2002).

Differences in the temporal profile of central responses when patients and controls are
subjected to the same amount of stimulation have also been reported. EEG components evoked by nociceptive stimuli have higher amplitude and longer duration (Lorenz et al., 1996) and habituation responses to nonpainful repetitive stimuli are delayed in FMS patients (Montoya et al., 2006).

In recent years, a number studies have employed MEG to investigate pain processing in the human brain (Maestú et al., 1998; Watanabe et al., 1998; Kanda et al., 2000; Kakigi et al., 2004). MEG offers a noninvasive method to study brain function with a temporal resolution in the scale of milliseconds (Hari et al., 1997). In contrast to EEG, MEG provides a non-reference based measurement of brain activity that is not distorted by the various biological tissues between the cortex and the scalp, or by extracellular volume conduction currents (Hämäläinen and Hari, 2002). To our knowledge, the present work is the first MEG study to investigate brain responses to subjectively matched non-painful and painful stimulation in fibromyalgia patients and controls. In previous MEG studies of fibromyalgia, responses to nonpainful stimulation were obtained instead (Montoya et al., 2004; Pollok et al., 2010).

Mechanical tactile stimulation of the tender point at the left lateral epicondyle, located near the elbow, was used to compare responses to painful and nonpainful stimuli in FMS patients and controls. Other approaches to evoke pain are laser stimulation (Huttunen et al., 1986; Kakigi et al., 1989; Miyazaki et al., 1994; Kanda et al., 1996; Kakigi et al., 1995; Hari et al., 1997; Granot et al., 2001), electrical stimulation (Robaina et al., 1989; Rushton, 2002) and making an incision during scanning with a non-magnetic scalpel (Burgmer et al., 2009).

The aim of the present work was to characterize differences in brain processing when stimulation is adjusted to elicit similar subjective levels of pain in both the FMS and the control group. While similar conditions were employed in the fMRI study by Gracely et al. (2002) the two techniques can provide complementary information, given their different spatiotemporal resolution and relationship to neuronal activity. In addition, in the previous fMRI study (Gracely et al., 2002), a statistical contrast between the two conditions was performed for each group separately, to assess how similar/dissimilar the patterns of active areas for the two groups are to one another. Here, the subthreshold condition was subtracted from the suprathreshold condition and, subsequently, a between-group statistical contrast was performed, to quantify differences in amplitude between active areas.

2. METHODS
Participants

Patients were recruited from Neuromadrid Medical Centre, Madrid, Spain. Inclusion criteria were the following: Patients had received a diagnosis of fibromyalgia according to the criteria of the American Association of Rheumatology, such that 11 or more tender points were positive to stimulation (Wolfe et al., 1990). They had been diagnosed at least 12 months prior to the beginning of the study. They were females between 18 and 60 years of age. Exclusion criteria were the following: Patients were asked to suspend their medication during the 2 months prior to scanning. This suspension included any medication except for, possibly, analgesics. Patients were not recruited if suspension of medication was not possible. Patients suffering from other medical condition different from fibromyalgia were also excluded. Inclusion and exclusion criteria for control subjects were the same except for the fibromyalgia diagnosis.

A total of 12 female patients and 11 female control participants, fulfilling the inclusion and exclusion criteria were recruited. Age, in years, was (M=36.1;SD=3.6) for patients and (M=28.4;SD=3.6) for controls, where M is the mean and SD the standard deviation. The clinical characteristics of the fibromyalgia patients are reported in Table 1.

TABLE 1 AROUND HERE

After scanning, 9 datasets from patients and 9 datasets from controls were kept for further analysis (see Data Analysis section). All participants signed an informed consent. All procedures were approved by the Review Board of the Technical University of Madrid and conformed to the Declaration of Helsinki.

Data Acquisition

Scanning was performed at the Dr Perez Modrego MEG Centre, Complutense University of Madrid, Spain, using a 148-channels (magnetometers) whole-head Magnes 2500 WH Magnetoencephalographer (4D Neuroimaging, Inc., San Diego, USA). The scanner was located in a magnetically-shielded room to reduce environmental noise. The signal was collected at the 148 magnetometers using a 1017.25 Hz sampling rate, and a real-time 0.1-200 Hz band pass filter was applied.

Stimuli

A pneumatic mechanical stimulation system (Maestù et al., 2009) was specifically developed to carry out this study. The operation of the system is as follows.
Computer-generated trains of pulses are received by a data acquisition board. The board communicates with a subsystem of valves which regulates the extension and retraction movement of a piston. The piston is secured to the arm of the patient with tapes. Information about the timing of the stimulation pulses is also sent from the acquisition board to the MEG system so that it can be coregistered with the brain responses.

The International Association for the study of Pain (IASP) guidelines (Merskey and Bogduk, 1994) were taken into account to define the delivered pressure range. The maximum pressure was chosen to be approximately twice the 4Kg/cm² IASP guideline pain threshold. This upper limit was set as a safety measure and to avoid causing excessive discomfort to participants. The exerted pressure was measured using a Force Ten™ Fdx Digital Force Gauge (Wagner Instruments, Greenwich, USA). Ten pressure steps increasing linearly from 1.9 Kg/cm² to 7.6 Kg/cm² were considered (Figure 1A).

**Protocol**

All subjects were examined by an expert physician to determine their status with respect to the inclusion and exclusion criteria. Next, the individual’s pain threshold was identified. The stimulation device was used to apply increasing amounts of pressure on the chosen tender point, at the left epicondyle, up to the specified upper limit (7.6Kg/cm²). Trains of pulses of one second duration and one second interpulse interval were employed (Figure 1B). Subjects were asked to report the level at which they started to feel pain. Four control participants failed to report feeling pain even at the maximum stimulation level. For these participants suprathreshold and subthreshold stimulation were set at level 10 and 8 respectively (Figure 1A).

Thereafter, a 5 minutes resting scan was obtained. Resting data was not analyzed in the present study. This was followed by a stimulation block with a pressure level two points above the individual’s pain threshold (suprathreshold condition). The stimulation block comprised 12 trains of 15 pulses each. Each pulse had a duration of one second and was followed by an interpulse interval of also one second. Train pulses were interleaved with rest periods lasting 15 seconds. A second stimulation block with the same structure followed, except that this time a pressure two points below the individual’s pain threshold level was applied (subthreshold condition).
Data analysis

The Fieldtrip toolbox (Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen, The Netherlands. http://www.ru.nl/neuroimaging/fieldtrip) was used for data analysis. Initially, the raw data was submitted to the MEG manufacturer's noise reduction procedure, which uses simultaneous recordings from nine reference channels, to minimize contributions from ambient magnetic noise. Thereafter, the maximum signal range (difference between maximum and minimum signal amplitude) across sensors was calculated for each trial. Trials with maximum signal range above $6 \times 10^{-12}$ Tesla were discarded. From the remaining trial distribution, the upper 20 percentile was further discarded to reduce the amount of artefacts in the recordings. Trials were then bandpass-filtered between 1 and 20 Hz, baseline corrected with respect to the $[-0.5: -0.05]$ seconds time window and averaged separately for each condition and participant. A minimum of 100 trials were employed for each average.

To check for robustness in responses, for each participant, epochs from all conditions were randomly divided into two groups, and the global field power was independently calculated for each group. Data from participants that showed similar profiles for the two groups of epochs were kept for further analysis. This led to nine patients and nine control participants being considered from the original twelve patients and eleven control participants. Such split-half comparisons have been used before, for example, to identify robust EEG components (Groppe et al., 2009).

Sensor level analysis

Subsequently, a sensor-level analysis was performed. The employed scanner comprises 148 magnetometers. While magnetometers are more sensitive to deep sources than other types of sensors, such as planar gradiometers, activity maxima and minima tend not to be directly above the sources. In contrast, planar gradiometers provide a spatial distribution for which the maximal signal is typically located above the source (Hämäläinen et al., 1993). For this reason, an estimate of the signal as recorded from planar gradiometers was calculated from the original magnetometer recordings (Bastiaansen and Knosche, 2000; Osipova et al., 2006; de Lange et al., 2008). Magnetometers measure the component of the magnetic field perpendicular to the surface defined by the sensor array whereas planar gradiometers measure the change in magnetic field in two orthogonal directions parallel to that surface. For each sensor, the two orthogonal components of the planar gradients were estimated using the signals from the neighbouring sensors. The modulus of the planar gradient vector was then obtained from these two components.

Next, evoked fields from each subject were realigned to a common sensor position in head
coordinates, as defined by averaging across the whole group. This is done to minimize the variability in the signal due to the fact that different subjects position their head differently inside the MEG helmet. An inverse linear source estimation in a sphere inside the helmet followed by a projection toward the common sensor array was used (Knosche et al., 2002). The event-related fields for the subthreshold condition were then subtracted from the event-related fields for the suprathreshold condition.

Source level analysis

Thereafter, a source level analysis was carried out. A minimum norm estimation (MNE) procedure commonly used in MEG source reconstruction (Hämäläinen and Ilmoniemi, 1994; Hauk, 2004) was applied to estimate the cortical origin of the brain responses. The minimum-norm solution finds the distribution of sources that best explains the sensor data while having minimal power. Since cortical pyramidal neurons are believed to be the main contributors to the MEG signal the dipoles of the source reconstruction model were restricted to a cortical surface extracted from a structural MRI (Dale et al., 2000). A tessellated cortical mesh template surface derived from the standard Montreal Neurological Institute (MNI) brain and implemented in SPM5 (www.fil.ion.ucl.ac.uk/spm/software/spm5) served as a brain model to estimate the current source distribution. The dipoles of the distributed source model are evenly placed at each node of the mesh representing the white/grey matter interface. The SPM5 template which we used contains 1504 dipole locations. This dipole mesh was used to calculate the forward solution using a spherical head model. As the magnetic field propagation is not distorted by the various tissue types of the head, a spherical head model is a good approximation to a realistic model in the case of MEG (Sarvas, 1987). The inverse solution (the estimation of the current source density based on the MEG topography) was calculated using the L2 minimum-norm solution (Hauk, 2004) using in-house MATLAB© code.

Statistical analysis

Nonparametric permutation testing (Holmes et al., 1996; Nichols and Holmes, 2002; Maris and Oostenveld, 2007) was applied to find spatiotemporal clusters with significant differences between groups. In brief, a between-group two-sample t-test was performed for each dipole (corresponding to a given location and latency). Neighbouring dipoles in space and time with a p-value below a certain value \( p_{\text{cluster}} \) were clustered together. For each cluster thus defined the sum of t-values across all dipoles comprising the cluster \( t_{\text{cluster}} \) was calculated. Subsequently, five-hundred surrogate t-maps were calculated by randomly dividing the participants from the two groups into surrogate groups matching the numbers in the original groups. This was done to obtain an empirical null distribution of the statistic.
of interest, where the null hypothesis is that both patients and controls are drawn from the same distribution. Clusters for each such surrogate map were obtained. From each map, the maximum and minimum tcluster values were included in the surrogate probability distribution. Thresholds were obtained from the quantiles of this distribution. For example, the 0.5th and 99.5th quantiles are used for a p-value of 0.01. This ensures that there is only a 1% chance that one or more clusters from the original statistical map will present differences above threshold due to statistical fluctuations, therefore correcting for multiple comparisons.

In the case of minimum norm solutions, for visualization purposes, a yellow circle marks the maxima and minima of statistical maps. A maximum of 4 maxima/minima per map are reported, no closer than 2 cm from each other and no less than 50% in amplitude with respect to the largest maximum/minimum for that map.

3. RESULTS

The pain threshold distribution for each group is reported in Figure 1C. An independent-samples t-test found significant difference ((t(16)=2.84, p<0.05) in pain thresholds, expressed in Kg/cm², between patients (M=5.5, SD=1.1) and control subjects (M=6.9, SD=0.88). Mean stimulation for patients was 4.4Kg/cm² in the subthreshold condition and 6.9Kg/cm² in the suprathreshold condition. Mean values for control subjects were 5.7Kg/cm² and 7.5Kg/cm² respectively.

FIGURE 1 AROUND HERE

Figure 2 shows the four significant clusters (p_{cluster}<0.005, p<0.01, corrected) arising from the statistical analysis at the sensor level. As described in the statistical analysis subsection in section 2, p_{cluster} is the statistical threshold associated with the definition of spatiotemporal activity clusters and p is the global p-value threshold after correction for
multiple comparisons. For each cluster, the left panel shows the signal time-course (suprathreshold-subthreshold) separately for patients and control subjects, averaged across the sensors comprising the cluster. Green windows denote time windows where the difference between patients and controls was significant. The sensors comprising the cluster are highlighted in the right panel. The topographic maps represent the signal averaged across the green time window and across all patients and controls.

Significant clusters were found around $t=70$ ms post stimulus onset over central and right anterior/central sensors (Cluster 1), around $t=170$ ms post stimulus onset over left anterior and left central sensors (Cluster 2) and around $t=420$ ms and $t=920$ ms post stimulus onset over anterior sensors (Clusters 3 and 4).

**FIGURE 2 AROUND HERE**

To examine the individual contribution of each condition to the four significant clusters found, Figures 3 and 4 provide the corresponding graphs for subthreshold and suprathreshold stimulation respectively. The signal amplitude is larger for control subjects than for patients in the subthreshold condition for these clusters (Figure 3). The opposite result is found in the suprathreshold condition (Figure 4), the differences being larger in the latter case.

**FIGURES 3 AND 4 AROUND HERE**

Differences in activity between patients and controls for the source reconstruction solutions are reported in Figure 5. First, the source-level response to subthreshold stimulation was subtracted from the response to suprathreshold stimulation for patients and controls independently and then a between-groups statistical contrast was calculated. Three significant clusters (A, B and C, $p<0.05$ corrected) emerge from the analysis. The curves indicate the minimum-norm activity for the corresponding cluster and group (Figure 5). The statistical maps show t-values averaged across the cluster time-window. Front, left and right views of the brain are provided. Yellow circles indicate local maxima. Talairach
coordinates and anatomical location of maxima, as described by the Talairach Daemon (Lancaster et al., 1997; Lancaster et al., 2000) are reported in Table 2. Maxima for cluster A (42-95 ms) are located in the contralateral (right) hemisphere in the Middle Frontal, Superior Temporal and Postcentral Gyri and in the Inferior Parietal Lobule. The two maxima in cluster B (150-220 ms) are in the ipsilateral (left) Middle Frontal Gyrus. Finally, maxima for cluster C (390-450) are located in the Middle and Medial Frontal Gyri and in the Posterior Cingulate.

FIGURE 5 AROUND HERE
TABLE 2 AROUND HERE

4. DISCUSSION

In the present study we obtained brain responses to subjectively matched non-painful and painful mechanical stimulation in fibromyalgia patients and controls. The main aim was to characterize differences in response to subjectively matched stimulation intensities both in space and time by taking advantage of the good combined spatiotemporal resolution of MEG.

The main result is that differences in brain responses between painful and non-painful mechanical stimulation were larger for patients that for controls (Figure 5). Such differences were significant in right somatosensory, temporal, parietal and prefrontal areas between 40-90 ms post stimulus onset, in left prefrontal areas (150-220 ms) and in right prefrontal areas (390-450 ms). These differences in activation profiles after source reconstruction were in good spatiotemporal agreement with differences found in sensor data (Figure 2). An additional significant cluster was found at sensor level between 920 and 940 ms over anterior sensors.

Two main pathways have been proposed to exist for pain processing. Lateral areas would mainly encode the sensory and discriminatory dimensions of the stimulus. Medial regions would be principally responsible for the affective, cognitive and evaluative aspects of processing (Albe-Fessard et al., 1985). According to this classification the first cluster (A) in Figure 5 would reflect mainly differences in sensory processing between the two groups, the third cluster (C) would indicate cognitive differences, and the second cluster (B) a combination of the two types of processes.
In terms of latency, components before 50ms are believed to encode physical stimulus parameters (Chen, 2000). Responses up to 130-150 ms have been linked to sensory registration and show no effect of attention/distraction (Garcia-Larrea et al., 1997). Components after 150 ms are likely to be primarily associated with pain perception as they can be modulated by attention, suggestion, hypnosis and mental stress (Chen et al., 1998). Therefore, according to their latency, cluster A is probably indicating differences in sensory encoding and clusters B and specially C differences in cognitive processing, in agreement with the previous analysis.

A previous EEG study showed an increased N80 component in FM patients using electrical stimulation (Diers et al., 2008), in agreement with the present work. This is one of the few studies where stimulation was adjusted to produce similar subjective pain, making it more directly comparable to the present study. Such increase in activity at early latencies was interpreted as reflecting enhanced sensory processing.

The first cluster occurs too early (40–90 ms) to reflect a nociceptive component of stimulation. Pressure stimulation typically stimulates A-beta, A-delta and C fibers. Stimulation of either A-delta or C fibers by noxious stimuli evokes cortical responses at later latencies. The earliest peak elicited by selective activation of A-delta fibers by laser takes place at approximately 160 ms post stimulus onset (Wang et al., 2007; Tsuji et al., 2006). Earlier responses can be detected at approximately 110 ms following stimulation but only when specific averaging techniques are employed (Wang et al., 2007). Selective stimulation of C-fibers with low intensity CO2 laser evokes a similar, but even later, response profile to A-delta stimulation, the first peaks being detected not before 500 ms following stimulus onset (Kakigi et al., 2005). Similarly, comparison of innocuous somatosensory and noxious cold stimulation reveals that the earliest response occurs at approximately 35 ms after stimulus onset for the former and after 160 ms for the latter (Maihöfner et al., 2002). Results of a conduction analysis suggested that somatosensory stimulation was transmitted by A-beta fibers while noxious cold stimulation was mediated by A-delta fibers (Maihöfner et al., 2002). It follows that the earliest cluster difference described in the present work most likely reflects a touch-related component transmitted by A-beta fibers rather than a nociceptive component mediated by A-delta or C fibers. Therefore differences at such early latencies argue for differences in processing between patients and controls of not only noxious components but also somatosensory components, after adjusting the pressure according to the individual’s pain threshold.

Pain perception is completed within the first 500 ms with cognitive evaluation taking place.
after 150 ms (Garcia-Larrea et al., 1997, Chen et al., 1998, Chen, 2000). Differences in prefrontal areas, as those shown in clusters A and B could reflect processes such as memory retrieval and fear association (Garcia-Larrea et al., 1997), pain emotion (Chen et al., 1998), coping and appraisal of emotionally relevant stimuli (Pessoa, 2009) and mechanisms of active control/reduction of pain perception (Lorenz et al., 2003).

No particular instruction was given to patients and participants regarding whether they should attend to or ignore the mechanical stimuli. Although it is not clear if a specific cerebral network dedicated to the modulation of pain by attention exists (Tracey et al., 2007) both the sensory and emotional dimensions of pain experience and the activity of a large number of pain-processing areas can be modulated by attention/distraction (Bantick et al., 2002, Ohara et al., 2004) after 150 ms post stimulus onset (Chen, 2000). In particular, medial prefrontal cortex has been related to anticipation of pain and dorsolateral prefrontal cortex to attention to pain (Gracely et al., 2004), and both regions show between-group differences in the present analysis. Therefore potential differences in attention between the two groups may have contributed to the profiles shown by clusters B and C. The activation of dorsal right parietofrontal areas have been classically involved in attentional orienting (Corbetta and Shulman, 2002). Alternatively/complementarily, the involvement of a right parietofrontal network in pain processing independently of the side stimulated has previously been interpreted in terms of automatic orienting towards relevant stimuli (Symonds et al., 2006). Nevertheless, previous fMRI results, such as reduced brain responses in anterior cingulate cortex in FMS, have been interpreted as evidence that patients pay less attention than controls to brief painful stimuli during experiments (Gracely et al., 2002), presumably because they are more accustomed to pain.

Behaviourally, fibromyalgia patients present with increased sensitivity to pressure stimulation as reflected in reduced pain thresholds (Harris et al., 2006). Central augmentation of pain processing may underlie this increased sensitivity as similar objective pressure to patients and controls results in greater cortical responses in patients as measured with fMRI (Gracely et al., 2002). An important complementary result of this study (Gracely et al., 2002) is that comparable subjectively painful stimulation evoked a much more similar activation pattern in patients and controls. While our results are consistent with the general thesis of augmentation of central pain processing in FMS we found not similar, but enhanced brain activity in FMS patients when stimulation was adjusted to produce similar subjective levels of pain, even if, objectively, pressure was higher for controls. This is in contrast with results from the fMRI study (Gracely et al., 2002) where patterns were mainly similar for both populations when similar subjective stimulation was applied, and, if anything, more areas were active in the control than in the patient population. It should be
noted though, that Gracely et al. (2002), performed a statistical contrast was between the painful and nonpainful condition separately for each group, and, subsequently, the patterns of active areas were compared qualitatively. This is different from the present analysis where signals from the subthreshold condition were subtracted from the suprathreshold condition and then a statistical contrast between the two groups was performed. Therefore, differences in amplitude between groups for a given area can be more readily assessed with the present analysis, while the previous work is more suitable to identify areas commonly coding for noxious signals in both groups. In this sense, results from the present study and the previous fMRI work are not necessarily contradictory, but rather they address a different question.

Furthermore, comparing results obtained with MEG and fMRI is not straightforward. The higher MEG temporal resolution results in a higher sensitivity in the present study to activity differences in the order of a few tens of milliseconds, as those presently found. On the other hand, due to its superior spatial resolution, the fMRI results are likely to be more precise with respect to activity localization, particularly for deeper sources (Hillebrand and Barnes, 2003). The relationship between the BOLD and MEG signals has been described as complex (Winterer et al., 2007). The closest correspondence seems to be with MEG oscillatory signals in the beta and gamma band (Winterer et al., 2007, Muthukumaraswamy et al., 2009, Stevenson et al., 2011). In the present work, evoked responses were obtained instead.

Increased responses to nociceptive stimuli could be the result of increased sensitization. Proposed mechanisms include increased excitability of spinal and supraspinal neurons, ongoing peripheral source of input from nociceptors, sensitized receptors on central nociceptive neurons and abnormal processing at supraspinal levels (Coderre et al., 1993; Staud et al., 2001). An alternative or complementary mechanism leading to enhancement in brain responses is reduction of inhibition (Montoya et al., 2006; Jensen et al., 2009). Recent fMRI evidence has pointed to altered cortical mechanisms for descending pain inhibition in FMS (Jensen et al., 2009). Additionally, an EEG study (Montoya et al., 2006) reported reduced habituation to repetitive nonpainful somatosensory stimulation in FMS patients which may reflect a lack of inhibitory control. In contrast, a recent MEG study (Pollok et al., 2010) found similar habituation effects in fibromyalgia patients and in controls during nonpainful tactile stimulation. While a decrease in habituation (Jensen et al., 2009; Montoya et al., 2006) could underlie the enhanced responses in the FMS group seen in the present work, some differences exist between the current study and the previous ones. First, MEG was employed here and fMRI was used in one of the previous studies (Jensen et al., 2009) and therefore differences in the origin and spatiotemporal resolution of the signals exist. As for the previous EEG study (Montoya et al., 2006), nonpainful stimulation was
applied in that work whereas in the present study enhanced responses in FM patients were found for painful stimulation. In addition, in the present work, stimulation for both populations was adjusted to evoke a similar level of pain while in the EEG study (Montoya et al., 2006) the same objective level of stimulation was used in both populations.

Alternatively to lack of inhibition, increased activity in frontal areas could reflect a higher demand on pain control areas in the case of patients. Increased activity in the Dorsolateral Prefrontal Cortex (DLPFC) has been shown to be associated with a decrease in perceived intensity and unpleasantness of nociceptive stimuli, and could therefore be involved in exerting active control on pain perception (Lorenz et al., 2003). Whether the present results reflect increased sensitization or lack of inhibition should be addressed with specific designs optimized to test for the effect of habituation/facilitation in the suprathreshold condition.

Limitations of the present study include the following. Differences in age between the two groups were statistically significant (t-test, t(16)=3.9, p<0.05). Nevertheless, the age range for the two groups [24-39] was short enough to suggest that the studied sample was sufficiently homogeneous. In fact, people in that age range are typically pooled together in pain studies (e.g. Riley et al., 2000).

Four control participants did not report feeling pain even at the maximum stimulation level. Future studies could benefit from recruiting a larger number of participants so that participants that fail to feel pain within the predetermined stimulation range can be excluded from the analysis. In general, future studies should consider enlarging the groups of patients and controls to be recruited so that active brain areas and their corresponding latencies can be identified more precisely. In this sense, the present work can be regarded as a pilot study for future investigations.

Pain thresholds were determined once in each individual using an intensity ascending train of pulses. It has been described that pain thresholds vary as a function of time and number of stimulation pulses received, and do so differently for patients and controls (Montoya et al., 2005). Future studies could consider determining pain thresholds as an average of several ascending and descending measurements and do so at different times during the course of the scan to better capture the dynamic nature of the process. This can be complemented further with the use of a visual analogue scale to determine pain ratings in the different conditions.

Finally, for all volunteers and patients the above-threshold stimulation block preceded below-threshold stimulation. While these made results more homogeneous across
participants it also implied that habituation/sensitization effects may have contributed to the within-group subtraction. While such effects deserve investigation in themselves, future studies could consider counterbalancing the presentation order across participants to improve the discrimination of the different components.

In conclusion, previous studies have found that stimulation adjusted to cause a similar level of pain in both FMS patients and controls activated overlapping groups of brain areas. This has been interpreted as evidence that the hypersensitivity to stimulation experienced by FMS patients could be the result of central pain augmentation. The present analysis, while consistent with the notion of central pain augmentation, reveals that the following areas are more activated in FMS than in controls: somatosensory, temporal, parietal and prefrontal areas at early latencies and prefrontal areas at late latencies. Therefore, brain responses to nociceptive stimuli are not uniquely determined by subjective sensation, but further differences exist between healthy subjects and fibromyalgia patients in both sensory and nociceptive components.

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FIGURE LEGENDS

Figure 1. A: Identification of pain threshold. Pressure levels used (in Kg/cm²). B: Ascending stimulation protocol to determine the pain thresholds. C: Distribution of pain thresholds for the patient and control groups.

Figure 2. Spatiotemporal clusters of significant (p<0.01, corrected) signal (suprathreshold-subthreshold) differences between patients and controls. For each cluster the left panel shows the signal profile (suprathreshold-subthreshold) averaged across the sensors comprising the cluster and group individuals (red=patients, blue=controls). Green line denotes cluster time window. Right panel: Highlighted are the sensors comprising the cluster. The underlying topographic map shows the cluster signal averaged across the signal time window and all patients and participants. (p_{cluster}<0.005)
Figure 3. Time-course for the subthreshold condition for the clusters defined in Figure 2. The left panel shows the signal profile for the subthreshold condition averaged across the sensors comprising the clusters defined in Figure 2 and all group subjects (red=patients, blue=controls). Green line denotes cluster time window. Right panel: Highlighted are the sensors comprising the cluster as defined in Figure 2. The underlying topographic map shows the cluster signal averaged across the signal time window and all patients and participants for the subthreshold condition.

Figure 4. Time-course for the suprathreshold condition for the clusters defined in Figure 2. The left panel shows the signal profile for the suprathreshold condition averaged across the sensors comprising the clusters defined in Figure 2 and all group subjects (red=patients, blue=controls). Green line denotes cluster time window. Right panel: Highlighted are the sensors comprising the cluster as defined in Figure 2. The underlying topographic map, shows the cluster signal averaged across the signal time window and all patients and participants for the suprathreshold condition.

Figure 5. Source reconstruction solutions. Statistical contrast of supratheshold-subthreshold minimum norm activity between patients and controls. The three significant clusters (A, B and C, p<0.05, corrected) are shown. Curves indicate minimum-norm activity for the corresponding cluster and group. Statistical maps show t-values averaged across the cluster time-window. Yellow circles indicate local maxima.
(A) Identification of pain threshold. Pressure levels used (in kg/cm$^2$). (B) Ascending stimulation protocol to determine the pain thresholds. (C) Distribution of pain thresholds for the patient and control groups.

Fig. 2.

Spatiotemporal clusters of significant ($p < 0.01$, corrected) signal (suprathreshold–subthreshold) differences between patients and controls. For each cluster the left panel shows the signal profile (suprathreshold–subthreshold) averaged across the sensors comprising the cluster and group individuals (red = patients, blue = controls). Green line denotes cluster time window. Right panel: highlighted are the sensors comprising the cluster. The underlying topographic map shows the cluster signal averaged across the signal time window and all patients and participants. ($p_{\text{cluster}} < 0.005$).
Fig. 3.

Time-course for the subthreshold condition for the clusters defined in Fig. 2. The left panel shows the signal profile for the subthreshold condition averaged across the sensors comprising the clusters defined in Fig. 2 and all group subjects (red = patients, blue = controls). Green line denotes cluster time window. Right panel: highlighted are the sensors comprising the cluster as defined in Fig. 2. The underlying topographic map shows the cluster signal averaged across the signal time window and all patients and participants for the subthreshold condition.
Fig. 4.

Time-course for the suprathreshold condition for the clusters defined in Fig. 2. The left panel shows the signal profile for the suprathreshold condition averaged across the sensors comprising the clusters defined in Fig. 2 and all group subjects (red = patients, blue = controls). Green line denotes cluster time window. Right panel: highlighted are the sensors comprising the cluster as defined in Fig. 2. The underlying topographic map, shows the cluster signal averaged across the signal time window and all patients and participants for the suprathreshold condition.
Source reconstruction solutions. Statistical contrast of suprathreshold–subthreshold minimum norm activity between patients and controls. The three significant clusters (A–C, $p < 0.05$, corrected) are shown. Curves indicate minimum-norm activity for the corresponding cluster and group. Statistical maps show $t$-values averaged across the cluster time-window. Yellow circles indicate local maxima.

Table 1.

Patient characteristics (group mean ± standard deviation). Pain and fatigue were measured with respect to a 0–10 visual analogue scale. Two patients had been diagnosed with depression and two with anxiety disorder.

<table>
<thead>
<tr>
<th>Time since diagnosis</th>
<th>Number of tender points</th>
<th>Daily sleep hours</th>
<th>Pain</th>
<th>Fatigue</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.9 ± 3.1 years</td>
<td>17 ± 1.5</td>
<td>6.6 ± 1.8</td>
<td>6.4 ± 1.4</td>
<td>7.1 ± 1.6</td>
</tr>
</tbody>
</table>

Table 2.
Talairach coordinates of contrast maxima between patients and controls from Fig. 5.

<table>
<thead>
<tr>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Cluster</th>
<th>Latency (ms)</th>
<th>Hemisp.</th>
<th>Lobe</th>
<th>Gyrus/lobule</th>
</tr>
</thead>
<tbody>
<tr>
<td>44.41</td>
<td>21.39</td>
<td>29.85</td>
<td>A</td>
<td>42–95</td>
<td>Right</td>
<td>Frontal</td>
<td>Middle Frontal</td>
</tr>
<tr>
<td>60.21</td>
<td>33.08</td>
<td>14.55</td>
<td>A</td>
<td>42–95</td>
<td>Right</td>
<td>Temporal</td>
<td>Superior Temporal</td>
</tr>
<tr>
<td>45.52</td>
<td>18.60</td>
<td>43.48</td>
<td>A</td>
<td>42–95</td>
<td>Right</td>
<td>Parietal</td>
<td>Postcentral</td>
</tr>
<tr>
<td>40.96</td>
<td>46.93</td>
<td>47.77</td>
<td>A</td>
<td>42–95</td>
<td>Right</td>
<td>Parietal</td>
<td>Inferior Parietal Lobule</td>
</tr>
<tr>
<td>−44.84</td>
<td>22.04</td>
<td>33.06</td>
<td>B</td>
<td>150–220</td>
<td>Left</td>
<td>Frontal</td>
<td>Middle Frontal</td>
</tr>
<tr>
<td>−39.94</td>
<td>37.51</td>
<td>−6.09</td>
<td>B</td>
<td>150–220</td>
<td>Left</td>
<td>Frontal</td>
<td>Middle Frontal</td>
</tr>
<tr>
<td>5.33</td>
<td>18.31</td>
<td>44.19</td>
<td>C</td>
<td>390–450</td>
<td>Right</td>
<td>Frontal</td>
<td>Medial Frontal</td>
</tr>
<tr>
<td>29.86</td>
<td>25.00</td>
<td>27.84</td>
<td>C</td>
<td>390–450</td>
<td>Right</td>
<td>Frontal</td>
<td>Middle Frontal</td>
</tr>
<tr>
<td>10.91</td>
<td>54.63</td>
<td>17.70</td>
<td>C</td>
<td>390–450</td>
<td>Right</td>
<td>Limbic</td>
<td>Posterior Cingulate</td>
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