Disinhibition of the primary somatosensory cortex in patients with fibromyalgia

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Abstract
Fibromyalgia (FM) is a chronic widespread pain condition linked to central sensitization. Altered excitability of sensorimotor cortex has been proposed as an underlying pathology of FM. This study aimed to investigate intracortical excitability of the primary somatosensory cortex (S1) and its potential role in clinical pain in patients with FM. Somatosensory evoked magnetic fields were recorded in 17 right-handed females with FM and 21 age-, sex- and handedness-matched healthy control subjects. Paired-pulse median nerve stimulation was delivered to the left and right wrist. We assessed the peak-to-peak amplitudes of the N20m–P35m and peak amplitude of each N20m and P35m component. Paired-pulse suppression (PPS) of the second response was quantified as the ratio of the amplitudes of the second to the first response. Patients with FM displayed significantly higher PPS ratio for the N20m–P35m in both hemispheres, indicating reduced intracortical inhibition in the S1. Notably, PPS ratio for the P35m was higher in patients with FM than in healthy controls, while no differences were apparent in PPS ratio for the N20m in both hemispheres. PPS ratios for both the N20m-P35m and the P35m in the left hemisphere were positively associated with the sensory pain on the short-form McGill Pain Questionnaire.

The present study demonstrated that intracortical inhibition in the S1 is compromised bilaterally in patients with FM, and the extent of disinhibition can be closely associated with increased clinical pain. Our results suggest that changes of intracortical inhibition of the S1 may contribute to the pathophysiology of FM pain.

Keywords: Fibromyalgia, Magnetoencephalography, Primary somatosensory cortex, Somatosensory evoked field, Paired-pulse suppression
1. Introduction

Fibromyalgia (FM) is a chronic pain syndrome characterized by the widespread pain and tenderness, often accompanied by fatigue, non-restorative sleep, and cognitive dysfunction [37,56]. Centrally mediated augmentation of sensory and pain processing is a well-established pathologic mechanism in FM [49,51,57]. It has been suggested that the augmented and ongoing nociceptive input to the brain leads to cortical reorganization and maladaptive neuroplasticity within the somatosensory and motor systems [25,34,35,41,44,50]. In a previous study using transcranial magnetic stimulation (TMS) of the motor cortex, it showed that the patients with FM had deficits in intracortical modulation possibly involving both γ-aminobutyric acid-releasing (GABAergic) and glutamatergic mechanisms [41]. Recently, we found that FM patients showed lower white matter integrity as measured by fractional anisotropy in the corpus callosum, the structure that enables interhemispheric communication between the sensorimotor cortices [22]. However, intracortical inhibitory function in the somatosensory system of the patients with FM has not been well understood so far.

The application of paired-pulse stimulation to a nerve trunk while recording somatosensory evoked potential (SEP) or magnetic field is considered a suitable method for investigating the intracortical inhibition in the somatosensory system [16,25,26,52]. The present study aimed to investigate intracortical inhibition of the primary somatosensory cortex (S1), as measured by paired-pulse suppression (PPS) ratio of the N20m–P35m response, in patients with FM by utilizing paired-pulse median nerve stimulation and magnetoencephalography (MEG). We hypothesized that intracortical inhibition of the S1 would be reduced in patients with FM, i.e. that patients with FM would demonstrate higher PPS ratios than healthy control (HC) subjects,
and that the higher reductions of inhibition would be associated with increased clinical pain. Also, since it has been suggested that the N20m and P35m components largely represent excitatory and inhibitory postsynaptic potentials at S1, respectively [15,16,28,55], we further examined PPS ratios separately for each of these components.
2. Patients and Methods

2.1. Participants

Nineteen right-handed female patients were recruited from the outpatient clinics of the Rheumatology Departments of the Seoul National University Hospital (SNUH) and Hallym University Sacred Heart Hospital. All examinations, except diagnosis and self-report questionnaires, were conducted at SNUH to ensure consistency of the results. Eligibility criteria for patients with FM were: 1) meeting the American College of Rheumatology 1990 criteria for primary FM [56]; 2) having a duration of widespread pain at least 3 months, but less than 10 years; 3) experiencing the pain intensity of at least 40, on the 0 to 100 mm pain visual analogue scale (VAS) over the past week; 4) aging between 30–60 years; and 5) willing to stop taking medications known to influence the somatosensory system (e.g. analgesics, antidepressants, and anticonvulsants) at least 3 days prior to the assessments. Patients were excluded if they had: 1) secondary FM associated with inflammatory arthritis; 2) a medical history of axis 1 psychiatric disorders (i.e., major depressive disorder, schizophrenia, substance abuse) or other disorders affecting the central nervous system (i.e. cerebrovascular accident, multiple sclerosis, Parkinson’s disease); 3) signs of peripheral neuropathy at the upper extremities (e.g. related to trauma, polyneuropathy); 4) concomitant acute pain in the upper extremities (e.g. due to injury); 5) hearing loss or use of hearing aids; 6) pregnancy or breastfeeding; or 7) contradictions for MEG (i.e. dental braces, permanent retainers) and/or magnetic resonance imaging (MRI) (i.e. pacemakers, cochlear devices, surgical clips, metallic implants, orthopedic pins) assessments.

Age-, gender-, and handedness- matched 21 HC participants were recruited by local advertisement. Exclusion criteria were same as for patients. The study protocol was approved by the institutional review board at SNUH and Hallym University Sacred Heart Hospital and was
conducted in compliance with the Declaration of Helsinki. All participants provided written informed consent.

2.2. Clinical assessments

For patients with FM, the disease duration (since the onset of widespread pain) and the number of tender points (as assessed by the treating rheumatologist using manual palpation upon inclusion) were registered. Additionally, the tender points count was (re-)assessed by a trained rheumatologist from the SNUH (H.W.K) in both FM patients and healthy subjects using a hand-held pressure algometer (Baseline Evaluation Instruments, Fabrication Enterprises, New York, USA). The Edinburgh Handedness Inventory [45], Beck Depression Inventory (BDI) [2], Beck Anxiety Inventory (BAI) [1], and the Fibromyalgia Impact Questionnaire (FIQ) [3] were assessed. The pain intensity for current and average, and the medication intake were assessed. Sensory and affective components of pain quality were assessed by the short-form McGill Pain Questionnaire (SF-MPQ) [39]. Demographic and clinical characteristics of the participants are presented in Table 1.

Table 1

2.3. Median nerve stimulation

The left and right median nerves were stimulated at the wrist with 0.2 msec of constant current pulses using bipolar electrodes in separate sessions. Electrical stimulations were sequenced with a STIM2 system (Neuroscan, El Paso, TX, USA) and delivered by using high voltage, constant current stimulator (Digitimer DS7AH, Digitimer Ltd, Hertfordshire, England).

Before the recordings, sensory and motor threshold were determined individually by
manually increasing the stimulator current output in the increments of 0.2 mA and 0.3 mA, respectively. We defined the sensory threshold as the lowest level of electrical stimulus intensity that produces the subtle tactile sensation on the skin of the wrist. The motor threshold was determined as the lowest level of stimulus intensity for eliciting a visible twitch of the abductor pollicis brevis muscle. All thresholds were determined twice and then averaged. The stimulus intensity was set to 130% of each subject’s motor threshold during the recording [14], where all participants did not feel any painful sensation. This was checked before and after MEG recordings.

2.4. Experimental conditions

Single-pulse stimuli and paired-pulse stimuli with an inter-pulse interval of 100 msec were presented alternatingly in one run [16]. The inter-stimulus interval between the single-pulse and paired-pulse stimuli was 3 sec. The subjects were instructed not to pay attention to the stimulus and to keep their gaze fixed on a cross, shown in the center of the display in front of them [16]. The order of the stimulation on the left and right median nerves at the wrist was randomized across subjects. The researcher (M.L) monitored both hands’ positions and stimulus-induced muscle twitches on-line via a video camera throughout the recordings.

2.5. MEG recordings

Neuromagnetic signals were recorded with a 306-channel whole-head neuromagnetometer (VectorView™, Elekta Neuromag, Helsinki, Finland) comprised of 102 identical triple sensors (two orthogonal planar gradiometers and one magnetometer) in a magnetically shielded room. During the MEG recordings, the subjects sat comfortably under the helmet-shaped sensor array
and were asked to keep their heads as still as possible. The exact location of the head with respect to the sensors was found by measuring the magnetic signals produced by electrical currents delivered to four head position indicator coils placed at known sites on the scalp. The location of head position indicator coils with respect to three anatomical landmarks, the nasion and two preauricular points, were measured by using a three-dimensional digitizer (FASTRAK™, Polhemus, Colchester, Vermont, USA), to allow alignment of the MEG and MRI coordinate systems. The x-axis passed through the two preauricular points with the positive direction to the right. The positive y-axis passed through the nasion, and the z-axis pointed upward. The high-resolution, T1-weighted, MR images were obtained from all subjects, except for one HC subject due to claustrophobia, using a Siemens 3T scanner (Siemens Magnetom TrioTim, Siemens, Erlangen, Germany).

The MEG signals were bandpass filtered (0.1–300 Hz) and digitized at 1 kHz. To reduce the environmental and biological noises, we applied the spatiotemporal signal space separation method using MaxFilter software version 2.2.10 (Elekta Neuromag, Helsinki, Finland) [29,53].

2.6. Data analysis

The analysis period of 400 ms included a pre-stimulus baseline of 100 msec. Epochs with amplitudes exceeding 3000 fT/cm for MEG channel or 150 µV for electrooculogram were excluded from the average. At least 120 artifact-free epochs were averaged for each single-pulse and first pulse of the paired-pulse. The data of 2 FM subjects were excluded from further analyses for the following reasons: excessive rejections of epochs due to signals contaminated by eye movements and the absence of MEG data with left median nerve stimulation.

After the paired-pulse stimulations with an inter-pulse interval of 100 msec, it was
shown that the response to the second pulse rides on the response to the first pulse (see Fig. 1B). Therefore, linear superposition effect was factored out, by subtracting the response to single-pulse stimuli from the response to paired-pulse stimuli, to assess “true” paired-pulse interaction (Fig. 1C) [13,14,25]. Based on these analysis procedures, the baseline corrected responses of N20m and P35m were obtained (Fig. 1C) [16].

The sources of the measured evoked responses to single electrical stimuli were modeled in the time domain as equivalent current dipoles (ECDs) [11]. This source analysis was performed blind to group status. The equivalent current dipole (ECD), which best explains the measured data, was determined at around 35 ms to obtain a S1 source with a high signal-to-noise ratio [28] by a least-squares search using a subset of 24 gradiometers over contralateral parietal areas. Those analyses led to the three-dimensional location, orientation, and strength of the ECD in a spherical volume conductor model of the head. For the subject for which no brain MRI was available, a sphere model was fitted to accurately digitized isotrak points [8] using MaxFilter software version 2.2.10 (Elekta Neuromag, Helsinki, Finland). Source identification for this subject was determined by considering coordinates, peak latencies, and orientations of the modeled sources. Only ECD with goodness-of-fit over 90% were accepted for further analysis. After identifying the single dipole, the entire analysis period and all channels were taken into account in computing the time-varying single dipole model. The location and orientation of the ECD were fixed. To validate the single-dipole model, measured MEG signals were compared to the responses predicted by the model. The estimated ECD for single-pulse condition were applied to the paired-pulse (Fig. 1B) and to the difference between paired-pulse and the single-pulse conditions (Fig. 1C) [28].

Fig. 1
For each subject, we analyzed the peak-to-peak amplitudes of the N20m–P35m, and peak amplitude of the each N20m and P35m component in response to the first pulse of the paired-pulse stimulation (A1). The peak-to-peak amplitudes of the N20m–P35m, and the peak amplitude of N20m and P35m components to the second pulse of the paired-pulse stimulation were obtained in the source waveform of paired-pulse minus single-pulses conditions (A2s). The PPS was then expressed as the ratio (A2s/A1) of the amplitudes of the second (A2s) to the first (A1) responses (PPS ratio for N20m–P35m, N20m and P35m). The higher ratios are associated with reduced PPS, while lower ratios are associated with stronger PPS.

Individual MRIs were spatially normalized in the Talairach coordinates using BrainVoyager QX software (version 1.10; Brain Innovation, Maastricht, The Netherlands) according to Lim et al. (2011) [27]. Source locations of the S1 responses in the head coordinates were transformed into Talairach coordinates using Brain Electrical Source Analysis software (version 5.1.8; MEGIS software, Munich, Germany). Twenty HC subjects whose MRI data were available are included in deriving the source location in the Talairach coordinates.

2.7. Statistical analysis

The PPS ratios of S1 response were compared using repeated measure analysis of variance (ANOVA) with hemisphere (contralateral to each hand) as within-subject factor and group (FM and HC) as between-subject factor. Source locations of the S1 responses were compared in each hemisphere separately by means of repeated measure ANOVA with coordinate (x, y and z) as within-subject factor and group (FM and HC) as between-subject factor. The Mauchly's test of sphericity was used to evaluate whether the sphericity assumption was violated. Our main question is to what extent intracortical inhibition of the S1 explains the variance of clinical pain
in FM patients. Therefore, linear regression analysis was performed with the PPS ratio as an independent variable, and the sensory and affective pain scores of the SF-MPQ and pain intensity on a VAS (past week) as a dependent variable. \( P \) values of <0.016 (0.05/3) were considered significant after Bonferroni correction. Since FM is a disorder with varying clinical characteristics (e.g., depressive and anxiety symptoms), we’d like to determine if these symptoms were reflected in our findings. Thus, the potential relationship between depressive or anxiety symptoms of FM patients and the PPS ratio were tested using Pearson's correlation in an exploratory fashion. \( P < 0.05 \) was taken as a prior significant threshold. We performed all statistical analyses using the SPSS 13.0 software package (SPSS Inc., Chicago, IL, USA).
3. Results

All group data are presented as mean ± standard deviations.

3.1. Stimulus intensity

Electrical sensory threshold was not different between groups in both left (FM for 1.63 ± 0.40 mA and HC for 1.54 ± 0.34 mA, \( P = 0.453 \)) and right hand (FM for 1.71 ± 0.45 mA and HC for 1.61 ± 0.31 mA, \( P = 0.464 \)). The stimulus intensities set at 130% of motor threshold for median nerve were not different between groups in both left (FM for 5.28 ± 1.20 mA and HC for 5.07 ± 1.17 mA, \( P = 0.590 \)) and right hand (FM for 5.28 ± 1.20 mA and HC for 5.57 ± 1.20 mA, \( P = 0.455 \)).

3.2. Paired-pulse suppression

Fig. 2 shows the cortical responses to single-pulse and paired-pulse median nerve stimuli in one representative HC and FM subject, respectively. The sources of the P35m response were located in the posterior bank of the central sulcus, corresponding to area 3b of S1. In HC subject, the amplitudes of the second response were markedly suppressed as compared to the first response in both hemispheres (A). In this subject, N20m–P35m PPS ratios for the left and right hemisphere were 0.578 and 0.617, respectively. In contrast, in patients with FM, amplitudes of the second response were comparable to the first response in both hemispheres (B). In this subject, N20m–P35m PPS ratios for left and right hemispheres were 0.773 and 0.983, respectively.

Fig. 2

The mean source locations of S1 in each hemisphere were superimposed on the standard brain (Fig. 3A). The results showed a main effect of coordinate (left, \( P < 0.001 \); right, \( P < 0.001 \), respectively), whereas no main effect of group (left, \( P = 0.431 \); right, \( P = 0.565 \), respectively)
and no coordinate X group interaction effect (left, \( P = 0.595 \); right, \( P = 0.369 \), respectively) were found both in the left and right hemispheres. In the FM group, the mean coordinates (mm) were \( x = -42.2 \pm 7.9, y = -21.4 \pm 7.8, \) and \( z = 48.3 \pm 5.7 \) for the left S1; \( x = 41.0 \pm 6.8, y = -18.2 \pm 7.6, \) and \( z = 50.1 \pm 6.2 \) for the right S1. In the HC group, the mean coordinates (mm) were \( x = -42.1 \pm 5.0, y = -24.2 \pm 7.2, \) and \( z = 48.3 \pm 5.3 \) for the left S1, and \( x = 42.2 \pm 4.5, y = -21.3 \pm 7.7, \) and \( z = 50.3 \pm 4.6 \) for the right S1. Fig. 3B shows the mean PPS ratios for the N20m–P35m in patients with FM and HC subjects. A repeated measure ANOVA showed a significant main effect of group (\( P = 0.009 \)), indicating that the PPS ratios were higher in patients with FM than in HC subjects. There was no significant main effect of hemisphere (\( P = 0.377 \)), nor was there a group X hemisphere interaction effect (\( P = 0.987 \)). As such, the mean PPS ratio for the N20m–P35m in both the left and the right hemispheres were higher in patients with FM than in HC subjects (Table 2). Notably, there was a significant main effect of group (\( P = 0.009 \)) for P35m component with no main effect for the hemisphere (\( P = 0.124 \)) and no significant group X hemisphere interaction (\( P = 0.879 \)) (Fig. 3D). In contrast, we did not find any significant effect of group (\( P = 0.890 \)), hemisphere (\( P = 0.698 \)), or their interaction (\( P = 0.837 \)) for N20m component (Fig. 3C).

With regard to the N20m–P35m amplitude, there was no main effect of group (A1, \( P = 0.740 \); A2s, \( P = 0.639 \), respectively) or hemisphere (A1, \( P = 0.623 \); A2s, \( P = 0.301 \), respectively) or any group X hemisphere interaction effect (A1, \( P = 0.756 \); A2s, \( P = 0.517 \), respectively) in response to both the first pulse of the paired-pulse stimulation (A1) and second pulse of the paired-pulse stimulation, after subtracting the response to a single-pulse (A2s) (Table e-1). With regard to amplitude of the first P35m after paired-pulse stimulation (A1), no significant main effect of group (\( P = 0.870 \)) or hemisphere (\( P = 0.257 \)) or any group X hemisphere interaction
effect ($P = 0.732$) were found. For amplitude of the second P35m after subtracting the response
to a single-pulse (A2s), ANOVA revealed a significant main effect of hemisphere ($P = 0.040$)
but no main effect of group ($P = 0.426$) or group X hemisphere interaction effect ($P = 0.562$).

Fig. 3

### 3.3. Correlation with symptom severity

Fig. 4 shows the relationship between the PPS ratio and clinical pain intensity in FM patients.

After correcting for multiple comparisons, a higher PPS ratio for the N20m–P35m in the left
hemisphere was significantly associated with higher clinical pain ratings in the sensory ($r^2 = 0.340, P = 0.014$), but not the affective ($r^2 = 0.262, P = 0.036$) dimensions of pain (Fig. 4A, Table e-2). Notably, a higher PPS ratio for the P35m was more closely associated with higher clinical pain ratings in the sensory ($r^2 = 0.410, P = 0.006$) dimension of pain (Fig. 4B). PPS ratio for P35m component was also positively associated with average pain intensity over the last week ($r^2 = 0.342, P = 0.014$) (Fig. 4C). In our exploratory analysis, we did not find any significant correlation between PPS ratios (N20m–P35m and P35) and BAI, and BDI scores in FM patients (all $Ps > 0.05$).

Fig. 4

### 4. Discussion

The present study is the first to demonstrate that in patients with FM, intracortical inhibition in
the S1 is compromised bilaterally and the extent of disinhibition can be closely associated with increased clinical pain.

The S1 has not received much attention compared to other brain regions involved in pain
modulation [17-21,30,48] as the pathophysiology of FM, which could be related to its well-
known role in early sensory discrimination. However, the pain-induced cortical reorganization and maladaptive neuroplasticity in the S1 has been suggested to be involved in the development and maintenance of chronic pain [25,35,44,50]. Reorganization of the somatotopic map within S1 was closely related to clinical pain in other chronic pain disorders such as phantom limb pain or complex regional pain syndrome (CRPS) [35,44]. In addition, a recent paired-pulse SEP study by Lenz et al. showed higher PPS ratio in the hemispheres contralateral to both the affected and unaffected hands in the patients with CRPS type I [25]. Previous FM studies have revealed evidence of cortical reorganization of the S1 due to central sensitization associated with persistent pain. Several electrophysiological and neuroimaging studies assessing brain activity during painful stimulus in patients with FM showed widespread augmented activation in brain regions including the S1 [4,7,33]. In addition, a recent resting-state functional MRI study reported increased spectral power in the S1, indicating the high level of baseline neural activity in FM patients [23]. FM has been also associated with structural reorganization, i.e. increased white matter fractional anisotropy, in the bilateral postcentral gyri [32]. In the present study, we observed a robust positive relationship between the reduced intracortical inhibition of the S1, and the sensory pain score on the SF-MPQ. Thus, cortical reorganization appearing as reduced intracortical inhibition of the S1 may be responsible for chronic pain in patients with FM. Because intracortical inhibition is known to have an important role in determining the size of the receptive field within S1 [12], reorganization of the somatotopic map measured by the distance between cortical representations within the S1 in patients with FM needs to be addressed in future studies. This cortical reorganization may explain the diffuse tenderness and widespread pain [49], as well as disturbances in body perception, such as reduction in awareness of limb positions and phantom swelling of the hands commonly reported by patients with FM [38].
The findings of disinhibition of the S1 in this study together with the changes in motor cortex excitability [41] and pain reduction after repetitive TMS over the motor cortex in the patients with FM [40,46] suggest that patients with FM exhibit altered excitability of the sensorimotor system in relation to chronic pain and its improvement. It has been suggested that the analgesic effects produced by motor cortex stimulation are related to the restoration of defective intracortical inhibition [24]. An interesting question is whether the abnormal intracortical inhibition in S1 would be reversed after repetitive TMS of the motor cortex.

So far, the mechanisms underlying PPS are not fully understood. Based on studies in animals, both presynaptic and postsynaptic GABAergic inhibition have emerged as important regulators of PPS [47,54]. In human subjects, paired-pulse SEP experiments have shown that PPS is generated rostral to the brainstem nuclei, presumably in the cortex [13]. Moreover, after the intravenous injection of the lorazepam, a GABA_A receptor agonist, the amplitude of P35m response was attenuated in the single-pulse condition and PPS of P35m component was strengthened (marginally significant) [16]. They further observed that P35m response did not recover in the 100 msec paired-pulse condition [16], which was similar to the behaviors of inhibitory postsynaptic potentials in intracellular recording [5]. Thus, taken together, our results of higher PPS ratio of the P35m component in FM patients compared to healthy subjects could be related to reduction of GABA-mediated inhibitory function in the S1. Recent studies using in-vivo magnetic resonance spectroscopy provided direct evidence of dysfunction of GABAergic system in FM [6]. Patients with FM had lower GABA level within the right anterior insula. In addition, GABA level within the right posterior insula was positively correlated with pressure pain threshold, suggesting alterations of inhibitory neurotransmission in FM. Additional assessments of GABA levels in the S1 might further contribute to the interpretation of our results.

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No group differences in amplitudes of the S1 response to single-pulse or first pulse of the paired-pulse stimuli are in line with results from a previous study that demonstrated no difference in the amplitude of the P50 response to first tactile stimuli of paired stimuli between FM patients and HCs [43]. In contrast, a recent MEG study by Maestu et al. reported that FM patients showed an augmented S1 response to subjectively-matched painful pressure stimulation on tender points [33]. They reasoned that enhanced responses in somatosensory and prefrontal areas could reflect an increased sensitization or lack of inhibition. Differences found between studies can be attributed two points; first, it might stem from the use of different stimulus sites (tender vs. non-tender points) and properties (painful vs. nonpainful). Second, responses to nonpainful sensory stimuli may not be augmented in early sensory cortices but may appear augmented during later stages of sensory integration as suggested by recent functional MRI study [31].

The experimental design of the present study was methodologically sound. First, the inter-pulse interval of 100 msec has been shown to be sufficiently short to identify a significant PPS in the S1 [10,16]. This was confirmed by our results, showing that the amplitude of the N20m and P35 components was successfully suppressed by paired-pulse stimulation in HCs. However, the higher PPS ratios in patients with FM were only found for P35m but not for N20m. This might be explained by the relatively short recovery cycle of N20m component, which reflects excitatory postsynaptic potentials [16,55]. Second, since the amplitudes of N20m and P35m components is known not to be affected by an inter-stimulus interval of 3 sec [55], preceding single-pulse stimuli would not affect the amplitude of the first S1 response to paired-pulse stimuli. Third, a recent study in HC subjects demonstrated no significant difference in PPS ratios when comparing different stimulus intensities (250% of the sensory threshold and 100%,
120%, and 140% of the motor threshold) [14]. Moreover, the stimulus intensity for the left and right median nerves was not different between the groups in our study. Thus, it is unlikely that the stimulus intensity might have affected between-group differences of PPS ratio. Lastly, although the subjects were instructed not to pay attention to the stimulus, the fixed order of stimuli could act as a possible confounder, in a way which affects anticipation of or attention to the next stimulus. However, in previous somatosensory evoked field studies, the amplitude of the S1 response was not changed by paying attention to the stimuli [9,36,42]. In addition, we observed that the amplitude of the N20m–P35m was not different between the response to single-pulse and that of the first pulse of the paired-pulse stimulation in both groups (Table e-1). Thus, we consider it is highly unlikely that the fixed order of stimuli affected the response to single-pulse or paired-pulse stimuli in individual subjects and altered the PPS ratio of the N20m–P35m response.

The present findings should be interpreted with caution. Although we tried to negate medication effects on cortical excitability by stopping all medications 3 days before the MEG recording this may not have been enough to rule out a potential effect. Even so, Mhalla et al. demonstrated that motor cortex excitability and intracortical modulation in patients with FM was not different between patients with and without taking medications [41]. Therefore, medication effects on cortical excitability in our study might be considered minimal as compared to disease effects. In the clinical correlation analyses between PPS ratio and clinical pain, a significant result was only found in the left hemisphere. We have no clear explanation for this finding at this stage. We speculated that this could be due to a hemispheric imbalance of disinhibition, since PPS ratio for the P35 component in the left and right hemispheres were correlated in the HC subjects ($r^2 = 0.323, P = 0.007$), but not in FM patients ($r^2 = 0.173, P = 0.096$). Changes of the
PPS ratio (A2s/A1) can be influenced by the amplitude of the second response or by the amplitude of the first response. In the present study, however, the amplitudes of N20m–P35m and P35m in response to the first pulse of the paired stimuli (A1) and the second pulse of the paired stimuli after subtracting the response to a single-pulse (A2s) were not different between FM patients and HC subjects. Taken together, our results cannot determine which amplitudes contribute to a reduced PPS ratio in patients with FM.

In summary, the present study demonstrated that intracortical inhibition in the S1 is compromised bilaterally in patients with FM, and the extent of disinhibition can be closely associated with increased clinical pain. Our results suggest that changes of intracortical inhibition of the S1 may contribute to the pathophysiology of FM pain.
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Figure legends

**Fig. 1.** Somatosensory evoked field from one gradiometer channel over the contralateral parietal area of the stimulated side (left) in a representative healthy subject.

The clear response deflections were found after single-pulse median nerve stimulation (A). The earliest downward deflection peaking at 20 ms (N20m) and the following deflections of opposite polarity peaking at 27 ms (P35m) were identified after the stimulus onset. After strong N20m and P35m responses to the first pulse of the paired-pulse stimulation, clear but weaker N20m and P35m responses to the second pulse of the paired-pulse stimulation was observed (B). The N20m and P35m responses to the second pulse of the paired-pulse stimulation were shown, after subtracting the response to a single-pulse from the paired-pulse stimulation (C). The black triangle indicates stimulus onset.

**Fig. 2.** Cortical responses to single-pulse and paired-pulse median nerve stimuli applied to left and right hands.

The location of the S1 source was superimposed on the brain MRI of a representative healthy control (A) and fibromyalgia subjects (B). White and black circles indicate location of the contralateral S1 response after right and left median nerve stimulation, respectively. Each left and right source waveform was generated in the contralateral S1 after the right and left median nerve stimulation, respectively. The vertical dotted lines indicate the stimulus onset. Open triangles = N20m; Filled triangles = P35m; S1 = primary somatosensory cortex.

**Fig. 3.** The mean locations of the primary somatosensory cortex responses superimposed on
a standard brain (A) and paired-pulse suppression ratio for N20m–P35m (B), N20m (C) and P35m (D) in fibromyalgia patients and healthy controls.

Red (fibromyalgia) and blue (healthy control) circles indicate location of the contralateral primary somatosensory response to left and right median nerve stimulation. Error bars indicate standard deviation. A1 = amplitude of the first response after paired-pulse stimulation; A2s = amplitude of the second response after paired-pulse stimulation, after subtracting the response to a single-pulse; PPS = paired-pulse suppression.

**Fig. 4. Relationship between paired-pulse suppression ratio and clinical pain in patients with fibromyalgia.**

The sensory dimension of clinical pain was measured by the short-form McGill Pain Questionnaire (SF-MPQ) (A and B). The average pain intensity over the last week was rated on a visual analog scale (VAS) (C). PPS = paired-pulse suppression.
Table 1. Demographic and clinical characteristics of study participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>FM (n = 17)</th>
<th>HC (n = 21)</th>
<th>Statistics</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, year</td>
<td>44.9 (8.7)</td>
<td>44.8 (8.2)</td>
<td>t = 0.03</td>
<td>0.98</td>
</tr>
<tr>
<td>Education, year</td>
<td>12.9 (2.2)</td>
<td>12.9 (2.7)</td>
<td>t = 0.04</td>
<td>0.97</td>
</tr>
<tr>
<td>Edinburgh score</td>
<td>81.1 (20.0)</td>
<td>84.6 (27.2)</td>
<td>t = -0.44</td>
<td>0.66</td>
</tr>
<tr>
<td>Duration of illness, month</td>
<td>37.0 (32.4)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Beck Depression Index score</td>
<td>19.0 (7.0)</td>
<td>2.8 (3.9)</td>
<td>t = 8.97</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Beck Anxiety Index score</td>
<td>22.1 (10.8)</td>
<td>1.8 (2.0)</td>
<td>t = 8.50</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TP manual</td>
<td>15.5 (1.8)</td>
<td>1.7 (2.3)</td>
<td>t = 20.48</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TP algometer</td>
<td>14.1 (3.9)</td>
<td>1.9 (1.7)</td>
<td>t = 12.88</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Pain VAS (average last week), mm</td>
<td>62.7 (16.2)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Pain VAS (day of assessment), mm</td>
<td>52.9 (22.8)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>FIQ</td>
<td>62.7 (13.9)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>SF-MPQ (sensory)</td>
<td>14.4 (6.7)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>SF-MPQ (affective)</td>
<td>5.6 (2.7)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>SF-MPQ (total)</td>
<td>20.1 (8.8)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Out of 19 patients involved in the study, 17 patients whose MEG data were analyzed are described. Additionally, analgesics were used by 71%, antidepressants by 71%, and anticonvulsants by 35% of these patients. All the values are expressed as mean (standard deviation). TP = tender point; VAS = visual analog scale (0 mm = no pain; 100 mm = worst pain imaginable); FIQ = Fibromyalgia Impact Questionnaire; SF-MPQ = short-form McGill Pain Questionnaire; NA = not applicable.
Table 2. Mean latencies, amplitudes, and paired-pulse suppression ratio of primary somatosensory cortex responses in patients with fibromyalgia and healthy control subjects

<table>
<thead>
<tr>
<th></th>
<th>FM (n=17)</th>
<th>HC (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left hemisphere</td>
<td>Right hemisphere</td>
</tr>
<tr>
<td></td>
<td>R1</td>
<td>R2s</td>
</tr>
<tr>
<td>Latency (msec)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N20m</td>
<td>19.4 (1.3)</td>
<td>18.3 (0.8)</td>
</tr>
<tr>
<td>P35m</td>
<td>30.2 (6.3)</td>
<td>30.0 (7.3)</td>
</tr>
<tr>
<td>Amplitude (nAm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N20m</td>
<td>25.8 (15.1)</td>
<td>15.7 (8.7)</td>
</tr>
<tr>
<td>P35m</td>
<td>41.7 (27.1)</td>
<td>22.9 (16.7)</td>
</tr>
<tr>
<td>N20m-P35m</td>
<td>67.5 (40.8)</td>
<td>38.6 (22.8)</td>
</tr>
<tr>
<td>PPS ratio (A2s/A1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N20m</td>
<td>0.629 (0.160)</td>
<td>0.609 (0.141)</td>
</tr>
<tr>
<td>P35m</td>
<td>0.561 (0.148)</td>
<td>0.604 (0.195)</td>
</tr>
<tr>
<td>N20m-P35m</td>
<td>0.586 (0.113)</td>
<td>0.603 (0.125)</td>
</tr>
</tbody>
</table>

All the values are expressed as mean (standard deviation). Statistical significances between fibromyalgia patients and healthy controls: *P < 0.05 (independent sample two-tailed t-test). R1 = response to first-pulse of the paired-pulse stimulation; R2s = response to the second pulse of the paired-pulse stimulation after subtracting the response to a single pulse; A1 = amplitude of the first response after paired-pulse stimulation; A2s = amplitude of the second response after paired-pulse stimulation, after subtracting the response to a single pulse; PPS = paired-pulse suppression; FM = fibromyalgia; HC = healthy control.
Table e-1. Mean amplitudes of the N20m−P35m.

<table>
<thead>
<tr>
<th></th>
<th>Fibromyalgia (n=17)</th>
<th>Healthy controls (n=21)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LH</td>
<td>RH</td>
<td>LH</td>
</tr>
<tr>
<td>sp A1</td>
<td>69.7 (43.5)</td>
<td>69.9 (42.0)</td>
<td>73.8 (23.0)</td>
</tr>
<tr>
<td>A1</td>
<td>67.5 (40.8)</td>
<td>69.5 (43.1)</td>
<td>71.7 (21.3)</td>
</tr>
<tr>
<td>A2</td>
<td>35.3 (22.6)</td>
<td>36.6 (23.6)</td>
<td>35.8 (15.6)</td>
</tr>
<tr>
<td>A2s</td>
<td>38.6 (22.8)</td>
<td>41.4 (25.6)</td>
<td>36.9 (13.6)</td>
</tr>
</tbody>
</table>

Mean values are expressed as mean (standard deviation). Independent sample two-tailed t-tests were used to compare the mean amplitudes of the N20m−P35 in each hemisphere; LH = left hemisphere; RH = right hemisphere; sp A1 = amplitude of the N20m−P35m response after single-pulse stimulation; A1 = amplitude of the first N20m−P35m response after paired-pulse stimulation; A2 = amplitude of the second N20m−P35m response after paired-pulse stimulation; A2s = amplitude of the second N20m−P35m response after paired-pulse stimulation, after subtracting the response to a single-pulse.
Table e-2. Regression results of relationship between paired-pulse suppression ratio and score of
SF-MPQ and pain VAS in patients with fibromyalgia

<table>
<thead>
<tr>
<th>SF-MPQ</th>
<th>PPS ratio for the N20m–P35m</th>
<th>PPS ratio for the P35m</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LH</td>
<td>RH</td>
</tr>
<tr>
<td>Sensory</td>
<td>0.340</td>
<td>0.014</td>
</tr>
<tr>
<td>Affective</td>
<td>0.245</td>
<td>0.043</td>
</tr>
<tr>
<td>Total</td>
<td>0.354</td>
<td>0.012</td>
</tr>
<tr>
<td>Pain VAS last week</td>
<td>0.145</td>
<td>0.132</td>
</tr>
</tbody>
</table>

LH = left hemisphere; RH = right hemisphere; PPS = paired-pulse suppression; SF-MPQ = short-form McGill Pain Questionnaire; VAS = visual analog scale.
A

Left hemisphere
Right hemisphere

B

C

D

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A  
\[ r^2 = 0.340 \]
\[ P = 0.014 \]

B  
\[ r^2 = 0.410 \]
\[ P = 0.006 \]

C  
\[ r^2 = 0.342 \]
\[ P = 0.014 \]