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Brain Activity Associated with Slow Temporal Summation of C-fiber Evoked Pain in Fibromyalgia Patients and Healthy Controls

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Abstract

Temporal summation of “second pain” (TSSP) is the result of C-fiber-evoked responses of dorsal-horn neurons, termed ‘windup’. This phenomenon is dependent on stimulus frequency (≥ 0.33 Hz) and relevant for central sensitization as well as chronic pain. Whereas our previous functional magnetic resonance imaging (fMRI) study characterized neural correlates of TSSP in eleven healthy volunteers, the present study was designed to compare brain responses associated with TSSP across these healthy participants and thirteen fibromyalgia (FM) patients. Volume-of-interest analysis was used to assess TSSP-related brain activation. All participants underwent fMRI-scanning during repetitive heat pulses at 0.33 Hz and 0.17 Hz to the right foot. Stimulus intensities were adjusted to each individual’s heat sensitivity to achieve comparable TSSP-ratings of moderate pain in all subjects. Experimental pain ratings showed robust TSSP during 0.33 Hz but not 0.17 Hz stimuli. When stimulus strength was adjusted to induce equivalent levels of TSSP, no differences in activation of pain-related brain regions occurred across NC and FM groups. Subsequently, the fMRI-data of both groups were combined to increase the power of our statistical comparisons. fMRI-statistical maps identified several brain regions with stimulus and frequency dependent activation consistent with TSSP, including ipsilateral and contralateral thalamus, medial thalamus, S1, bilateral S2, mid- and posterior insula, rostral and mid-anterior cingulate-cortex. However, the stimulus temperatures necessary to evoke equivalent levels of TSSP and corresponding brain activity were less in FM patients. These results suggest that enhanced neural mechanisms of TSSP in FM are reflected at all pain related brain areas, including posterior thalamus, and are not the result of selective enhancement at cortical levels.

Keywords

Temporal summation; Second pain; Windup; fMRI; Fibromyalgia

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1. Introduction

Fibromyalgia (FM) is a chronic pain syndrome whose pathogenesis is only partially understood. Accumulating evidence has linked FM pain to abnormal central processing at spinal, and supraspinal levels, including ineffective descending controls (Kosek and Hansson 1997; Lautenbacher and Rollman 1997; Desmeules et al. 2003). Although peripheral sensitization also may play a role in FM pain (Littlejohn et al. 1987; Sann and Pierau 1998; Al Allaf et al. 2001; Granot et al. 2001; Staud 2007c), the relevance of this pain mechanism for this chronic pain syndrome is currently unknown.

Abnormalities of spatial and temporal pain summation mechanisms have been described in FM using psychophysical methods (Staud et al. 2001; Desmeules et al. 2003; Julien et al. 2005). In particular, temporal summation of “second pain” (TSSP), termed ‘windup’ appears to be a clinically relevant mechanism for central sensitization and chronic pain (Price et al. 1977). TSSP is considered to be the result of C-fiber-evoked responses of dorsal horn neurons and is dependent on stimulus frequency (≥ 0.33 Hz). Several studies have emphasized the important role of TSSP for chronic musculoskeletal pain, in particular FM (Staud et al. 2001; Staud et al. 2003a; Staud et al. 2003b; Desmeules et al. 2003).

We recently completed a functional magnetic resonance imaging (fMRI) study in twelve healthy pain-free volunteers (NC) utilizing volume-of-interest analysis to measure brain responses associated with TSSP (Staud et al. 2007a). All study participants underwent fMRI scanning during repetitive heat pulses to the right foot at 0.33 Hz and 0.17 Hz. Stimulus intensities were adjusted to each individual’s heat sensitivity to achieve comparable TSSP ratings of moderate pain at 0.33 Hz. Using fMRI statistical maps, we identified several brain regions showing stimulus- and frequency-dependent activation consistent with TSSP including those commonly identified in pain imaging studies [contralateral thalamus (THAL), S1, bilateral S2, anterior and posterior insula (INS), mid-anterior cingulate cortex (ACC), and supplemental motor areas (SMA)].

The present study extends our previous work in NC and examines the brain responses associated with TSSP of thirteen FM patients. We compared the TSSP related brain activity of NC and FM patients and utilized a new method of analyzing TSSP associated brain activation. Whereas our previous fMRI analysis used simple contrasts of blood-oxygen-level-dependent (BOLD) activity related to 6- and 2-heat pulse trains to confirm TSSP, our present approach modeled all pulses simultaneously within each train of pulses. By subjectively equating the magnitude of TSSP across NC and FM patients through adjustment of individual stimulus temperatures, we also wanted to test whether FM patients required *lower stimulus temperatures* to evoke the same magnitude of TSSP and TSSP associated brain activity compared to NC subjects. This group difference would provide independent confirmation of increased TSSP sensitivity in FM patients (Staud et al. 2004).

2. Materials and Methods

We recruited thirteen middle-aged healthy pain-free female subjects [mean age (SD): 42.9 (10.3) years] using advertisements posted throughout the University of Florida, Gainesville and seventeen female FM patients [43.4 (7.5) years] from the local community or FM support groups. All FM patients fulfilled the 1990 American College of Rheumatology Criteria for FM (Wolfe et al., 1990). Informed consent was obtained from all subjects and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. The University of Florida Institutional Review Board approved the procedures and protocol for this study. Six subjects (NC: 2; FM: 4) were excluded from the fMRI analysis because of incomplete scans. All participants were right handed and included 22 Caucasians, one African-American and one

Asian subject. Prior to testing, all subjects underwent a clinical examination and were excluded from the study if they had abnormal findings (except asymptomatic osteoarthritis of the hands or knees) or other findings unrelated to FM. Use of analgesics, including non-steroidal anti-inflammatory drugs (NSAID) and acetaminophen, was not allowed during the study. All subjects were asked to discontinue analgesics for the duration of five drug half-lives before testing, except narcotics which had to be stopped at least two weeks prior to study entry. Low dose muscle relaxants and/or amitriptyline (<10 mg/day) were permissible during the study for treatment of FM-related insomnia.

All pre-menopausal subjects were tested during the luteal phase of their menstrual cycles as determined by menstrual history.

2.1 Experimental Design

The purpose of this study was to compare pain-related brain activity associated with C-fiber activation and TSSP in NC and FM subjects. We elicited TSSP by applying repetitive 47 to 51°C heat pulses (see below) to the plantar surface of the right foot of study subjects (for more details, see Staud et al. 2007a). Subjects usually report moderate pain and TSSP during repetitive pulses at frequencies of ≥ 0.33 Hz but not at 0.17 Hz (Staud et al. 2001; Staud et al. 2006). The painful sensations evoked by such repetitive stimuli display latencies consistent with C-fiber transmission (Staud et al. 2001; Staud et al. 2007b). They are well separated from early sensations such as 1st pain and can be easily detected by study subjects. Importantly, heat pulses applied to the glabrous skin of the foot can evoke brief latency sensations of weak pain or warmth, but do so only during the first two pulses of a heat pulse train (Vierck et al. 1997; Price et al. 1977; Price et al. 1994). Thus, pain ratings and measures of neural activity, related to repetitive heat pulses almost exclusively result from C-fiber input and TSSP.

2.2 Ratings

2.2.1 Ratings of Experimental Pain—A standardized numerical pain scale (NPS) was utilized for rating the magnitude of painful sensations produced by thermal stimulation as described previously (Staud et al. 2007a). The scale ranged from 0 to 100, in increments of 5, with verbal descriptors at intervals of 10. Previous experience with the scale has shown that increments of 5 provide appropriate resolution for discriminable levels of late sensation intensity from threshold to nearly intolerable levels (Vierck et al. 1997; Staud et al. 2001). This numerical scale has been found to be particularly advantageous for pain ratings during a series of repetitive stimuli (Vierck et al. 1997).

2.2.2 Ratings of Somatic Pain and Anxiety—Numerical scales (0 – 100) were used for ratings of somatic pain and anxiety during the experimental protocol. Although the subjects were required to be pain free at enrollment somatic pain ratings were obtained before and during the scanning session to capture possible new onset pains like back pain, headache, etc. The scales were anchored on the left with “no pain/anxiety at all” and on the right with “the most intense pain/anxiety imaginable”. Ratings of somatic pain and anxiety were obtained before and after each scanning run.

2.3 Thermal probe

An MR-compatible Peltier thermode with a 3×3 cm (9 cm²) contact surface (TSA-2001, Medoc Advanced Medical Systems, Ramat Yishai, Israel) was used for the thermal stimuli during the experiments. For TSSP testing the probe was firmly attached to the plantar surface of each subject's right foot by Velcro® straps. The right foot was used for TSSP testing in all subjects.

2.3.1 Heat Pulses—During the fMRI scanning session, each subject received four different types of heat pulse trains (Figure 1). Two and six repetitive heat pulses at 0.17 and 0.33 Hz were used to test the dependence of TSSP on stimulus number and frequency, respectively, i.e. TSSP was expected to increase dependent on number and frequency of the stimuli. All heat pulse trains were counterbalanced to control for order effects. The interval between stimulus trains was always 2 min. For each heat pulse, the temperature of the thermal probe increased from baseline to peak temperature by 8°C/sec, before returning to baseline at a rate of 8°C/sec. The duration of each heat pulse was always 3 sec (1.5 sec rise time; 1.5 sec return time). The probe temperature was adjusted to each individual's heat pain sensitivity which was determined in psychophysical experiments prior to fMRI scanning (see 2.3.2). During these experiments the probe temperature was determined that achieved maximal thermal TSSP ratings of 45 ± 10 NPS units after six heat pulses at 0.33 Hz.

2.3.2 Adjustment of Thermal Stimuli to Each Subject's Pain Sensitivity—Pain sensations from heat stimuli vary as a function of each subject's peripheral and/or central sensitivity, which influences the rate of TSSP and its decay. Because this variability is likely to affect comparisons of TSSP between NC and FM subjects, we needed to provide measures of TSSP sensitivity across all subjects. Thus, to measure TSSP sensitivity, we determined the unique stimulus temperature for each subject during preliminary experiments that resulted in final heat pulse ratings of 45 ± 10 NPS units of the last (6th) stimulus at 0.33 Hz. Stimulus intensities resulting in moderate heat pain ratings (45 ± 10 NPS units) were chosen to avoid altered peripheral and central pain sensitivity of study subjects during repeated trials. During preliminary trials, 0.33 Hz stimulus trains were always started at 47°C (peak pulse temperature), and, if necessary, the temperature was subsequently raised until subjects achieved NPS ratings of 45 ± 10 (NPS_{max}) after 6 pulses. This stimulus intensity was used for subsequent TSSP testing in the MR scanner.

Due to technical constraints of the thermal stimulator used for the TSSP experiments, the maximal rise and fall of probe temperatures were limited to 8°C/sec. Thus, necessary adjustments of peak temperatures always required similar changes in baseline temperatures for TSSP heat stimuli. Although somewhat unusual, this heat pulse design was necessary to elicit similar magnitudes of TSSP in all subjects.

2.3.3 TSSP Training Sessions—All subjects were trained to attend to and rate late sensations evoked by repetitive thermal stimulation of the plantar surface of the right foot during at least two separate training sessions. Details of similar TSSP procedures have been previously reported in detail (Staud et al. 2001; Staud et al. 2004; Staud et al. 2007a). Prior to the testing sessions, a technician who was unfamiliar with the hypotheses of the experiment instructed each subject to provide ratings of the intensity of any delayed pain sensations produced by repeated heat pulses to the plantar surface. Study subjects were told that they *may or may not* feel sensations of heat pain during each pulse and that a late sensation of heat or heat pain would likely be perceived 1 to 2 sec after each heat pulse. They were also asked to pay attention to and provide numerical ratings of the magnitude of the late sensation, which could *increase or decrease with stimulus repetition*. Before each fMRI session, the subjects practiced rating of late thermal sensations inside and outside the scanner to familiarize themselves with the NPS.

Similar to our previous fMRI study (Staud et al., 2007a), all subjects were able to easily distinguish early from late pain sensations and to selectively rate the latter. Because glabrous foot areas are most distant from the spinal cord and brain, delayed pain sensations (2nd pain) are well separated from early sensations such as 1st pain. Furthermore, the ability of FM and NC to distinguish the two pains has been established in previously published experiments (Staud et al. 2007b). Heat pulses applied to the glabrous skin of the foot evoke early sensations

of weak pain or warmth and these early sensations diminish somewhat and do not temporally summate during trains of heat pulses (Staud et al. 2001; Staud et al. 2007b). Thus, TSSP and associated neural activity results from repeated C-fiber input (Price et al. 1977; Staud et al. 2007b).

2.3.4 Pain Ratings of Thermal Stimuli Before and During fMRI Scans—After resting comfortably on the scanner table the subjects were moved into the MR scanner to acclimatize themselves to the scanning environment. Subsequently, two six pulse trains of thermal stimuli at 0.33 Hz and 0.17 Hz were applied to the right foot without image acquisition. In contrast to subsequent scanning runs, the subjects were instructed to rate the pain intensity after each heat pulse using the NPS. This procedure served as an experimental check for the subsequent fMRI experimental manipulations in which the subjects reported the remembered heat pain ratings of the last heat pulse of a train after each scanning run. Specifically, the subjects were instructed before each scanning run to pay attention to the pain intensity of each stimulus and remember the number rating of the last stimulus of each heat pulse train which always occurred 30 sec before the end of each run (see Figure 1). After each scanning run the subjects were immediately asked to provide the remembered number rating of the last stimulus.

2.4 Questionnaires

All subjects were instructed to complete Beck's Depression Inventory (BDI) (Beck and Beamesderfer 1974) and the Spielberger State/Trait Anxiety Questionnaires (Spielberger et al. 1983). The BDI is a self-administered 21 item self-report rating inventory measuring characteristic attitudes and symptoms of depression. Scores can range from 0 – 63. A score of 19 and higher is indicative of clinical depression. Spielberger's State/Trait Anxiety Inventory consists of 20 items each that ask how a person feels now, and reflects situational factors that may influence anxiety levels. Scores range from 20 to 80 and the higher the score the greater the level of anxiety. These questionnaires were only used to characterize the study subjects.

2.5 fMRI Scanning

2.5.1 fMRI Mock Scanning—To familiarize the subjects with the scanning environment, all participants completed heat TSSP trials in a mock scanner that simulated the conditions of the Siemens Allegra Head Scanner. Recorded scanner noise was played during the training sessions at realistic intensity levels. All subjects underwent at least two training sessions inside the mock scanner to reduce environment-related anxiety and to familiarize themselves with the stimulation protocols.

2.5.2 TSSP fMRI Scanning Design—During the scanning session the subjects were comfortably placed on the scanning table in the supine position with their knees supported by a wedge cushion. Their head positions were fixated with foam pillows to prevent excessive motion. Ear plugs and ear phones were used for noise protection as well as communication with the subjects. All subjects completed 32 functional scanning runs, each comprising eight two-pulse trains and eight six-pulse trains at stimulus frequencies of 0.33 Hz and eight two-pulse trains and eight six-pulse trains at stimulus frequencies of 0.17 Hz (Figure 1). At the end of each stimulus train scanning was continued for 30 sec to capture delayed pain-related brain activity and allow the hemodynamic response to return to baseline levels. The duration of each functional run was always 88 sec regardless of the number or frequency of the applied heat stimuli. Due to the two different frequencies used for testing, two and six pulse trains started either at 16 sec and 32 sec (0.17 Hz), or 60 sec and 76 sec (0.33 Hz), respectively, after the beginning of the scanning run (Figure 1). Therefore the last stimulus of every heat pulse train and similarly pain ratings occurred at exactly the same time point for all functional runs, regardless of the number and frequency of the applied heat stimuli. Using the NPS, the subjects were instructed to rate the pain of the last stimulus of each train and then retain this rating in

memory until the end of each functional run. After each run, the subjects were immediately asked to report the number rating of the last stimulus. Before and after each run the subjects also rated clinical pain and anxiety using the NPS.

2.5.3 Image Acquisition—MRI data were acquired on a research-dedicated head scanner (Siemens Allegra, 3.0 T) using a standard head RF coil. Prior to functional scanning, high-resolution 3D anatomical images were acquired using a T1-weighted MP-RAGE protocol (128 1-mm axial slices; TR = 2000 ms, TE = 4.13 ms, FA = 8°, matrix = 256 × 256 mm, FOV = 24 cm) to enable transformation and localization of functional data into Talairach space (Talairach & Tournoux, 1988). Functional images were acquired in 32 axial slices oriented parallel to the anterior commissure–posterior commissure (AC–PC) line using a T2*-weighted gradient echo planar imaging (EPI) sequence [Repetition time/echo time (TR/TE = 2000 ms/30 ms), flip angle (FA) = 90°, field of view (FOV) = 24 cm, matrix = 64 × 64; voxel size = 3.75 mm × 3.75 mm × 3.8 mm; slice gap 0.4 mm]. Each functional run lasted 88 sec, resulting in 44 volumes of fMRI data. To reduce T1-saturation effects the first two volumes of each run were discarded at the scanner and two additional volumes were discarded during pre-processing, leaving a total of 40 volumes per functional run and 320 volumes per condition.

2.5.4.1 Functional MRI Data Reduction: Data were analyzed with a Xeon dual-processor 3.4 GHz workstation using BrainVoyager (BVQX 1.8 - Brain Innovation, Maastricht, the Netherlands; <http://www.brainvoyager.com>). Image pre-processing consisted of rigid-body 3D motion correction using trilinear interpolation, slice-scan time correction with sinc interpolation, spatial smoothing with a 4-mm full-width at half maximum (FWHM) Gaussian kernel, voxel-wise linear detrending, and high-pass temporal filtering to remove nonlinear drifts below 3 Hz. Functional data were co-registered to 3D anatomic volumes before being transformed into standard space using the standard 8-parameter landmark method of Talairach and Tournoux (1988). During spatial transformation, functional voxels were interpolated to a resolution of 1 mm³.

2.5.4.2 Whole Brain Analysis by Random Effects GLM: Our previous work (Staud et al. 2007a) demonstrated the ability to use random effects (RFX) General Linear Models (GLM) to identify brain regions where TSSP was significantly convolved with the hemodynamic response function (HRF). The RFX analysis first estimates within subject contrasts (Level-1), before subjecting significant voxels to group analyses (Level-2). Time-course (see Figure 1) and parameter specification have been described elsewhere (see Staud et al. 2007a, for details).

For all the analyses, the BOLD responses to the heat pulse trains of each condition were modeled. However, the BOLD responses to the last pulse of each train were our primary variables of interest. To test possible group differences in TSSP between FM and NC, we used an RFX analysis of the whole brain to a) estimate individual activation patterns related to maximal TSSP (0.33 Hz - 6 pulse trains vs. 0.33 Hz - 2 pulse trains, i.e., Level 1), and b) compared group level activation between NC and FM subjects (Level 2). This analysis tested the hypothesis that the BOLD activation patterns for the NC and FM subjects were not statistically different when experimental pain levels were equated across groups. For this study, the RFX Level-1 parameter estimates were used in the Level-2 statistical analyses. The resultant Level-2 statistical parametric maps (SPM) were thresholded using a false-discovery-rate (qFDR < 0.05) (Genovese et al. 2002), which is commensurate with a corrected $p < 0.00001$, and a *minimum* spatial extent of 50 contiguous voxels (i.e. 50 μL). SPMs were then overlaid on an averaged 3D anatomical volume, in standardized Talairach space, which was used for localization.

2.5.4.3 Identification of Volumes of Interest (VOIs): TSSP is dependent on stimulus number as well as frequency and occurs reliably in NC and FM subjects during heat pulse trains of $\geq$

0.33 Hz (Staud et al. 2001; Staud et al. 2006). We have previously reported on TSSP-related brain activity in discrete volumes of interest (VOIs) in healthy pain-free individuals (Staud et al. 2007a). The ANCOVA module of BrainVoyager QX (version 1.8), allowed us to specify a 2×2 ANOVA for stimulus frequency (0.17 and 0.33 Hz) and train length (2 and 6 pulses). Rather than focus on the response magnitude for the last heat stimulus as in the RFX analyses, the overall response from all stimuli were used in the ANOVA. However, rather than adopting the default, polynomial contrast approach, to identify main- or interaction- effects we took a more conservative statistical approach. To ensure the identification of TSSP-related VOIs, we specifically compared the brain activity of 0.33 Hz 6-pulse trains (TSSP_{max}) with the linear combination of all other (3) stimulus conditions.

VOIs were chosen if active voxel clusters in resultant SPMs met the following criteria: a) cluster integrity for both individual (Level 1) and group (Level 2) contrasts; b) voxels exceeded the significance test threshold of $FDR < 0.02$, $p < 0.0005$; c) cluster volumes were at least 100 μL (i.e. 100 contiguous voxels); and d) the center-of-mass gravity for the voxel cluster was in an identifiable, conceptually meaningful brain region.

2.5.4.4 Group Comparisons Used for VOI Analyses: Using a voxel-wise RFX-GLM, 19 VOIs were identified following the procedures outlined above (see Table 1). The VOIs identified in the previous step were used in our third statistical analysis. Hence the VOIs were used as cortical masks which served several functions. Pragmatically, the VOI analyses allowed us to examine activation patterns in pain-related regions only, eliminating the need for additional whole-brain analysis. Statistically, the VOI analyses increased sensitivity in detecting differences in BOLD signals by reducing the overall number of comparisons (see Table 2).

3.0 Results

3.1 Ratings of Somatic Pain and Negative Affect

The healthy subjects reported no somatic pain before and during the fMRI scans. In contrast the FM subjects' overall pain VAS scores were 2.9 (1.2) before and 3.7 (1.4) after the scanning procedures ($t(22) = 1.24$; $p > .05$). NC mean (SD) Beck Depression Inventory (BDI) score was 2.6 (3.9) (range 0 – 10) and their Spielberger State/Trait Anxiety scores were 29.7 (9.1) and 45.6 (6.6), respectively. BDI scores of FM subjects were 13.2 (9.5) and their state/trait anxiety scores were 33.9 (3.4) and 43.1 (3.4), respectively. Independent t-test of BDI scores showed that FM patients' ratings were significantly higher than NC ($t(27) = -2.9$; $p < .05$). The BDI scores of FM patients indicate low levels of depression and are well below the cut-off for major depressive episodes (BDI score > 20) (Geisser et al. 1997). Similarly, Spielberger State/Trait anxiety scores were low in FM patients and not statistically different from NC ($p > .05$).

3.2 Experimental Pain Ratings

The subjects' remembered pain ratings of the last stimulus of two pulse and six pulse heat trains at 0.33 Hz and 0.17 Hz are shown in Figure 2. A mixed model ANOVA with diagnostic group (2), stimulus number (2), and stimulus frequency (2) as independent variables showed a significant main effect for frequency ($F(1,21) = 60.6$; $p < .001$) and stimulus number ($F(1,21) = 94.3$; $p < .001$). There was a significant interaction effect of frequency $\times$ stimulus number noted ($F(1,21) = 35.2$; $p < .001$). There was, however, no significant main effect for diagnostic group ($p > .05$). FM subjects showed a tendency for greater TSSP in the 0.33Hz condition compared to NC ($F(1,21) = 6.5$, $p = .04$). However, as noted above the main effect for group was non-significant. Furthermore, the difference in pain ratings in the 0.33 Hz condition was relatively small (6 NPS units) and well below that previously demonstrated to be sufficient to show brain activation differences (Staud et al. 2007). In addition, the interaction between

stimulus number $\times$ diagnosis was not significantly different ($p > .05$). These findings indicate that TSSP was observed in NC and FM subjects and that its magnitude was dependent on stimulus number and frequency. Although, TSSP of FM subjects was slightly greater than NC at 0.33 Hz, this finding was not unexpected and within our experimental design. The heat pulse intensity for sensitivity adjusted TSSP was a-priori selected for each individual subject to achieve maximal TSSP ratings of 45 ± 10 NPS units at 0.33 Hz. This design took into account the observed increase of peripheral/central sensitivity of study subjects due to multiple repetitions of stimulus trains required for fMRI analysis. The small differences in TSSP ratings between NC and FM subjects, however, did not result in group differences of TSSP-related brain activity (see 3.4).

3.3 Heat Pulse Intensities Used for TSSP Stimuli

TSSP stimuli were adjusted to each subject's heat sensitivity prior to scanning. The average peak stimulus temperature necessary to achieve 45 ± 10 NPS units during scanned TSSP runs was $49.8 (0.9) ^\circ\text{C}$ and $48.6 (1.6) ^\circ\text{C}$ for NC and FM subjects, respectively. An independent t-test showed that these mean peak temperatures used for TSSP testing of FM and NC were statistically different ($t(26) = 2.49$; $p = .019$).

3.4 Whole Brain fMRI Comparisons of NC and FM Subjects

Whole brain voxel-wise contrasts of activation patterns for TSSP (0.33 Hz - 6 pulse trains vs. 0.33 Hz - 2 pulse trains), failed to identify any significant differences in TSSP-related brain activity between the NC and FM subjects ($\text{FDR} < .05$; $t(22)$; $p(\text{Bonf}) < .69$), indicating that our approach of equating pain sensitivity and thus TSSP across subjects and groups had been successful. Thus all subsequent analyses were carried out using the pooled data from both groups. This approach substantially increased our statistical power to detect variations in TSSP-related brain activity as well as determine the time course of TSSP-related BOLD activation within multiple VOI.

3.5 Volumes of Interest Analysis

Given the lack of statistical differences for diagnostic group comparisons of the whole-brain analyses, the functional data of NC and FM subjects were pooled to increase construct specificity and contrast sensitivity for the VOI analyses. Using stringent selection criteria for VOIs (see 2.5.4.3) we identified 19 TSSP-related brain regions common to both FM and NC subjects (see Table 1 for VOI details). Random Effects VOI-GLMs were used to test differences in BOLD (associated with the last pulse) between the paradigm associated with TSSP (0.33 Hz, 6 pulses) and all other conditions in 19 brain regions (Table 1). These regions were contained within the 16 general ones hypothesized to show increased activity during the experimental conditions as described in Staud et al., 2007. Consistent with the psychophysical data of TSSP, there was significantly greater activation seen during the 6-pulse condition at 0.33 Hz than all other conditions combined ($\text{FDR} < .02$; $t(22)$; $p(\text{Bonf}) < .0006$) (Figure 3). These differences occurred for all hypothesized regions (left post-THAL, right post-THAL, left S1, left S2, right S2, anterior and post-INS, right ACC). Thus, stimulus frequency (0.33 Hz $>$ 0.17 Hz) and number of stimuli (6 $>$ 2 stimuli) were important determinants of VOI activation, consistent with the psychophysical results.

3.5 BOLD Activity Time Course During TSSP Trials

The BOLD signals increased during TSSP trials with 6 heat pulses at 0.33 Hz and remained elevated for more than 40 sec in all VOIs after the last heat stimulus (Figure 4 & 5). In contrast 6-pulse trials at 0.17 Hz resulted in a much smaller increase of BOLD activity lasting for approximately 10 sec (Figure 4 & 5). Thus the observed brain activity associated with TSSP trials is unlikely to represent a BOLD artifact. In addition, the increase in BOLD activity during

0.33 Hz pulses was reminiscent of the prolonged pain sensations intrinsic to TSSP as described in previous psychophysical experiments (Vierck et al. 1997; Staud et al. 2001).

Thus this evidence suggests that the observed activation of cortical and subcortical areas of NC and FM subjects was related to TSSP, including the BOLD activity time course and the highly significant association of VOI-BOLD responses with maximal TSSP (0.33 Hz, 6th pulse) compared to all other stimulus conditions.

4. Discussion

Using fMRI, we identified the brain responses associated with TSSP of NC and FM patients during repeated heat-pulses to the glabrous surface of the foot. TSSP was associated with activation in several brain areas known to receive input from ascending spinal pathways and sites involved in pain-related somatic sensation, cognition, and affect. When the magnitude of TSSP was adjusted to each individual's pain sensitivity, as done in our study, no group differences in pain-related brain activity were apparent on functional MRI images. Both the magnitude and time course of TSSP-related brain activity were similar in NC and FM patients (Figure 4 & 5). However, FM patients required lower stimulus intensities for sensitivity adjusted TSSP, indicating that their TSSP mechanisms necessitate less primary afferent input compared to NC, but are not qualitatively different. Brain regions showing TSSP-related activity included those involved *at all levels* of somatosensory afferent processing, (post-THAL, mid-THAL, S1, S2, mid and post-INS), pain-related cognition (dorsal ACC, inferior frontal gyrus, medial frontal gyrus) and affect (rostral ACC and ACC area 24).

Since brain activity associated with both sensory and cognitive/affective aspects of pain was similar in NC and FM patients, the increased TSSP sensitivity of FM patients did not result from mechanisms that selectively enhance brain activity at higher cortical areas, as suggested by the results from other studies (Mertz et al. 2000; Gracely et al. 2002; Cook et al. 2004). Our results, however, cannot exclude that the enhanced TSSP of FM patients was at least partially determined by a) primary afferent or dorsal horn sensitization, b) enhanced descending facilitation, c) decreased descending inhibition, or d) an unknown widely distributed mechanism within the brain.

4.1 TSSP Sensitivity is Enhanced in FM Patients

The average peak temperature of heat pulses used for TSSP testing was significantly lower for FM patients (48.6°C) than NC subjects (49.8°C). This result indicates that lower stimulus intensities were sufficient in FM patients to evoke the same magnitude of TSSP and TSSP-related brain activity. Although this difference may not appear to be large, it likely corresponds to a distinct increase in responses of populations of C nociceptive afferents that are very sensitive to changes of this magnitude, especially within this temperature range [e.g. approximately 30% reduction (Beitel and Dubner, 1976)]. Enhanced sensitivity to TSSP, using this "response dependent" method, is consistent with previous studies utilizing the same stimulus levels across NC and FM patients (Staud et al. 2001; Price et al. 2002; Staud et al. 2003c; Staud et al. 2006).

4.2 Neural Responses Associated with TSSP

In our study, trains of identical heat pulses were used to evoke C-fiber dependent pain summation. It is important to recognize that single heat pulses as used in our experiments, evoke only very weak 'first pain' or warmth, whereas repetitive heat-pulses (6-pulse trains at 0.33 Hz) result in moderate pain (approx. 50 NPS units) and TSSP (Price et al. 1978; Vierck et al. 1997). In addition, the heat-pulse trains utilized in our experiments were particularly

useful to examine C-fiber pain and its summation. The same temporal parameters that evoke TSSP were used to identify neural responses in multiple brain areas.

We have previously reported TSSP-related brain activity in NC (Staud et al., 2007a) making use of the same data set of pain-free subjects as in our current analysis. Whereas our previous analysis utilized simple contrasts to confirm TSSP by comparing maximal brain activity associated with brief (2-pulse) and extended (6-pulse) heat pulse trains at two stimulus frequencies (0.33 Hz and 0.17 Hz), the present study employed analysis of variance (ANOVA) to simultaneously model all heat pulses and stimulus frequencies tested. This approach provided a more powerful and refined characterization of neural activity associated with TSSP (see Methods). As hypothesized, TSSP-related brain activation occurred in somatosensory (THAL, S1, S2, ant-INS and post-INS), cognitive–evaluative/affective (ACC, PFC), and pain-modulating brain regions (rostral ACC).

We acknowledge that some contributions to TSSP-related brain activity may result from non-neuronal carryover of BOLD during multiple stimuli, similar to that found in previous studies (Huettel et al. 2004). Thus, it is inevitable that the BOLD signal at the end of a six pulse 0.33 Hz train will be larger than at the end of a six pulse 0.17 Hz train or at the end of a 0.33Hz two pulse train. However, we think that the temporal summation of BOLD responses observed in our study mostly results from neuronal activation. This view is supported by the fact that non-neural components of BOLD activity decrease within approximately 6–12 seconds after repetitive mechanical or visual stimulation (Huettel et al. 2004), whereas TSSP-related BOLD responses remained well above baseline levels for more than 40 seconds after termination of the six pulse 0.33 HZ train but not other stimulus trains (Figure 4 & 5). In addition, a recent fMRI study demonstrated strong associations between BOLD activity and action potential activity within the same cortical area when repetitive acoustic stimuli were used (Mukamel et al. 2005).

4.3 TSSP Mechanisms are Similar in FM and NC Subjects

The demonstration of TS of C-fiber evoked responses within brain areas known to be important for pain processing further supports their role in the encoding of TSSP. Both our previous fMRI study of NC (Staud et al., 2007a) and the present report show that neural activity associated with TSSP is not limited to somatosensory processing areas (e.g., S1, S2, post-INS) but extend to brain regions more directly involved in cognitive evaluations and affect (rostral ACC, dorsal ACC, SMA, medial frontal cortex) (see Table 1). The similarities of activations in all of these areas across FM patients and NC subjects are noteworthy.

Furthermore, FM patients required lower intensities of repetitive heat stimuli than NC to evoke similar pain intensity and brain activity. These results are in partial agreement with Gracely et al., (2002) who reported that FM patients needed lower levels of pressure stimuli to their thumbnails than NC to evoke similar brain activation and pain. In contrast to our study, however, these investigators reported thalamic activation only in NC but not in FM patients and the activated cortical areas of these groups only partially overlapped. Their results could be interpreted to reflect selective enhancement at cortical levels rather than a general increase in afferent processing. The differences between their results and those of the present study are puzzling and may be related to the use of different pain stimuli (single pressure stimuli versus repetitive heat pulses). At least for TSSP, our present results show both strong and approximately equal levels of posterior thalamic activation in both NC and FM subjects (Figure 3), suggesting that the abnormal TSSP sensitivity of FM patients is mainly the result of differences in afferent processing of pain.

4.4 Limitations

Although interesting and possibly important, we did not compare brain activation and pain under conditions wherein the same stimulus intensity was applied to both FM and NC subjects. Previously it has been reported that FM patients show greater brain activation in a larger number of areas in comparison to NC subjects when identical pressure stimuli are applied to the thumb (Gracely et al. 2002). To make similar comparisons with TSSP, however, is extremely challenging, because the use of fixed temperature heat stimuli increases risks of FM patients to intolerable TSSP sensations. In addition, the time required for testing this paradigm would have extended the scanning sequences considerably. Because it was already difficult for FM patients to remain still in the scanner during our study, we opted to address this issue in a separate investigation. Another limitation of our study is that it did not distinguish between bottom-up and top-down abnormalities as contributing to brain mechanisms of enhanced TSSP in FM, as pointed out above.

5. Conclusions

TSSP is dependent on frequency as well as number of stimuli and occurs predictably when heat pulses are applied to the skin at ≥ 0.33 Hz. The increasing brain activation of NC and FM patients related to repetitive heat stimuli closely resembles their psychophysical TSSP data as shown by the BOLD curves in our study (Figure 4 & 5). When sensitivity adjusted heat stimuli are used in NC and FM patients, TSSP-related brain activity is not different between groups. The combination of our results and others (Gracely et al., 2002) indicate that FM patients have widespread hyperalgesia that includes the hands as well as the feet and is associated with increased activation in pain processing areas of the brain.

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Figure 1.

Repetitive thermal stimuli at 0.33 Hz and 0.17 Hz were used to image TSSP-related brain activation. Previous psychophysical studies have shown that stimulus frequencies of ≥ 0.33 Hz resulted in robust TSSP whereas frequencies of < 0.33 Hz were only minimally effective. During scanning runs, two and six heat stimuli at 0.33 Hz and 0.17 Hz were applied to the right plantar surface, respectively. We previously used this format of stimulation for the NC subjects (Staud et al. 2007a). To remain consistent, the same paradigm was used for the FM subjects in the current study. Each run lasted for 88 sec and was comprised of discarded acquisitions/baseline, repetitive thermal stimuli at 0.17 Hz or 0.33 Hz, and a 30 sec follow-up period. Although the four different trains used during scanning runs varied in the timing of the first stimulus, the last stimulus of each train always occurred at the same time point of a run. Duration of discarded acquisitions/baselines is indicated by hatched lines; stimuli at 0.33 Hz or 0.17 Hz are shown by solid black lines; the 30 sec follow-up periods are represented by solid grey lines.

Figure 2.

Average ratings (SD) of maximal experimental pain during TSSP trials at 0.17 Hz and 0.33 Hz in NC and FM subjects. All subjects underwent eight repetitions of two pulse or six pulse trains at either frequency in the scanner. All experimental pain ratings were obtained at the end of each scanning run using the NPS. The experimental stimuli were adjusted outside the scanner to each individual's heat sensitivity to obtain TSSP ratings of 45 ± 10 NPS units after 6 stimuli at 0.33 Hz. inside the scanner, however, the mean

Figure 3.

Brain activity related to TSSP stimuli in sagittal, axial, and coronal sections (Panel A and B). Data of NC and FM subjects were combined for this analysis. The fMRI images depict the difference in brain activation between the sixth (last) stimulus at 0.33 Hz and all other stimulus conditions (including the 2-pulse trains at 0.33 Hz and 2- and 6-pulse trains at 0.08 Hz). The brain activity in all a-priori selected VOIs was significantly greater after six pulses at 0.33 Hz compared to all other conditions at, including S1, ACC, THAL, INS, and cerebellum ($p < .006$). Statistical maps are overlaid on structural MRIs averaged across all study participants. (THAL: thalamus; S1: somato-sensory cortex 1; ACC: anterior cingulate cortex; INS: insula).

Figure 4.

Representative samples of BOLD signal time courses related to six TSSP stimuli at 0.33 Hz (black lines) and 0.17 Hz (grey lines) of NC and FM subjects. VOIs: left rostral ACC; left THAL. Arrows indicate the duration of six heat stimuli at 0.33 Hz (black arrow) and 0.17 Hz (grey arrow). Residual brain activation after 0.33 Hz 6-pulse trains took more than twice as long to resolve as expected. In contrast residual brain activity after 0.17 Hz 6-pulse trains resolved within the expected time period. Similar BOLD time courses were observed at all VOIs. Signal intensity time courses were averaged across all significant voxels within statistically-identified clusters. TSSP: temporal summation of second pain; THAL: thalamus; BOLD: blood oxygen level dependent.

Figure 5.

Representative samples of BOLD signal time courses related to six TSSP stimuli at 0.33 Hz (black lines) and 0.17 Hz (grey lines) of all subjects (NC and FM) combined. VOIs: left rostral ACC; left THAL. Arrows indicate the duration of six heat stimuli at 0.33 Hz (black arrow) and 0.17 Hz (grey arrow). Brain activity was most pronounced during six heat stimuli at 0.33 Hz. Similar BOLD time courses were observed at all VOIs. Signal intensity time courses were averaged across all significant voxels within statistically-identified clusters. TSSP: temporal summation of second pain; THAL: thalamus; BOLD: blood oxygen level dependent

Table 1
Common TSSP-related VOIs for pain-free NC and FM subjects

Hemisphere	Structure	Talairach Coordinates			Cluster Volume (μL)
		X	Y	Z	
Left	THAL, Lateral Posterior Nucleus	-15	-21	13	2325
Right	THAL, Lateral Posterior Nucleus	16	-20	13	1651
Left	THAL, Medial Dorsal Nucleus	0	-16	9	396
Right	S1, BA 2, 3, 5	3	-38	62	101
Left	S1, BA 3, 5	-4	-33	70	171
Right	S2, BA 40	50	-37	26	2092
Left	S2, BA 40	-51	-44	29	1077
Left	S2, BA 40	-51	-29	24	846
Left	Inferior Parietal Lobule, BA 40	-51	-30	25	911
Left	Post INS	-33	-18	8	770
Left	Mid INS	-34	-2	8	984
Left	Dorsal ACC, BA 31	-12	-31	44	1984
Left	Rostral ACC, BA 24	-3	11	31	2060
Left	Mid ACC, BA 24	-3	-12	42	1589
Right	Precentral Gyrus, BA 13	48	-10	12	307
Right	Inferior Frontal Gyrus, BA 47	45	19	-4	156
Left	Medial Frontal Gyrus, BA 9	-8	43	17	295
Right	Superior Temporal Gyrus, BA 13	47	-42	25	1436
Midline	Cerebellum	3	-55	-22	473

BA: Brodman's area; THAL: thalamus; S1: somato-sensory cortex 1; S2: somato-sensory cortex 2; INS: insula; ACC: anterior cingulate cortex

Table 2

Between groups contrast of TSSP-related VOIs

Hemisphere	Structure	Between Groups <i>p</i> -value
Left	THAL, Lateral Posterior Nucleus	0.667
Right	THAL, Lateral Posterior Nucleus	0.368
Left	THAL, Medial Dorsal Nucleus	0.058
Right	S1, BA 2, 3, 5	0.294
Left	S1, BA 3, 5	0.567
Right	S2, BA 40	0.902
Left	S2, BA 40	0.640
Left	S2, BA 40	0.626
Left	Inferior Parietal Lobule, BA 40	0.525
Left	Post INS	0.845
Left	Mid INS	0.232
Left	Dorsal ACC, BA 31	0.520
Left	Rostral ACC, BA 24	0.398
Left	Mid ACC, BA 24	0.795
Right	Precentral Gyrus, BA 13	0.457
Right	Inferior Frontal Gyrus, BA 47	0.132
Left	Medial Frontal Gyrus, BA 9	0.936
Right	Superior Temporal Gyrus, BA 13	0.561
Midline	Cerebellum	0.518

BA: Brodman's area; THAL: thalamus; S1: somato-sensory cortex 1; S2: somato-sensory cortex 2; INS: insula; ACC: anterior cingulate cortex